Production of soymilk yoghurt fermented with

*Bifidobacterium longum BB536*

A Dissertation Submitted to Sudan University of Science And Technology in partial fulfillment for the degree of B.Sc. in food Science and Technology.

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Dedication

To our beloved mothers and fathers
For their support and encouragements
, to our brothers and sisters
and all our friends
to all teachers in different education levels
Acknowledgement

Special praise and thanks to Almighty ALLAH for granting us health and strength to complete this work. In a special way, I would like to thank our supervisor Dr. Barka Mohamed Kabeir for excellent guidance.

We would like to all staff at Department of Food Science and Technology for their Kind Cooperation. Special thanks to Dr. Salma Elzein, Ustaz Eihab Hatem and Algile Omer. Our deeply thanks go to our family. Also we thank everyone who deserves our thanks.
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Abstract

The objective of this study was to produce yoghurt from soymilk fermented with *Bifidobacterium longum* BB536. Yoghurts were formulated from 100%cow milk (A), 100% soymilk (B) and mixture of 50%cow milk with 50% soymilk (C). Different analysis were conducted for raw materials and processed yoghurts including proximate composition (moisture, protein, fat, Ash), pH, Total solids, microbiological analysis (strain BB 536 number), and sensory properties. Soymilk contained higher levels of protein, fat, and ash as compared to cow milk. Analyses on growth of strain BB 536 in different yoghurt showed survival up to the second weeks refrigeration storage fulfilling requirements of probiotic which at least 6 LogCFU/ml. The results of sensory evaluation revealed that panelists accepted yoghurt B and C as similar to control yoghurt A. Therefore, processing of yoghurt from soymilk was successful. Same time soymilk yoghurt has high nutritive value than cow milk yoghurt in addition to high level of probiotic strain BB 536.
مختصر الدراسة

الهدف من هذه الدراسة هو إنتاج الزبادي من حليب الصويا المتخمر بالبكتيريا الصديقة. تم تجهيز الخلطات للزبادي من 100% حليب الأبقار (A) وخليط من حليب الأبقار بنسبة 50% مع لبن صويا بنسبة 50%. أجريت التحاليل المختلفة للمواد الخام والزيادي شملت التحاليل التقريبية (البروتين – الدهن – الرماد) والأس الهيدروجيني. الجوامد الصلبة الكلية، التحاليل الميكروبية لسلالة البكتيريا الصديقة BB والخصائص الحسية. إحتوى لبن الصويا على محتوى عالي من البروتين، الدهون والرمال مقارنة مع حليب الأبقار. أجري تحليل نمو وحياة سلالة باكتيريا BB B باستمرار حتي الأسبوع الثاني من التخزين في الثلاجة والذي يفي بمتطلبات البكتيريا الصديقة والتي لا تقل عن 6 Log CFU/ml. أظهرت نتائج التقييم الحسي القبول للعينتين (A) و C كمثيل للعينة المرجعية. لذلك، إنتاج زبادي من لبن الصويا كان ناجحا. وفي نفس الزبادي المنتج من لبن الصويا إحتوى على قيمة غذائية عالية مقارنة بزبادي لبن الأبقار بالإضافة لإحتوائه على مستوى عالي من سلالة البكتيريا الصديقة BB.
CHAPTER ONE

INTRODUCTION

Soyabean have an exceptional nutritional and functional food profile. Soy-food are considered to be nutritious and healthy based on their nutrient composition (UNUCED, 2016). It is an excellent source of protein and oil of good quality. It contains about (43%) protein, (21%) carbohydrates, (5%) minerals, (8%) moisture, (20%) fat, (4%) fiber (Ganshrao, 2016). Soybean is rich in calcium and vitamin B12. Tocopherols are an important constituent of soy oil, due both to the vitamin E supplied for human nutrition and their antioxidant properties (CNUCED, 2016).

Soymilk is an aqueous, white, creamy extract produced from soybeans which is similar to cow milk in appearance and consistency. It is a highly nutritious which contains protein, fat, carbohydrates vitamins and minerals. Soymilk beverage worldwide is credited to health benefits such as: low cholesterol and lactose, its ability to reduce bone loss and menopausal symptoms, prevention and reduction of heart diseases and certain cancers. As this drink is cholesterol free and low in energy, it could enhance health benefits in terms of reducing body weight and blood lipids (kohli et.al., 2017).

Milk (from cow) is a mixture of fat, protein, carbohydrate, minerals and vitamins. The fat and protein exists as a colloidal suspension in a mixture of water which contains water soluble carbohydrate and minerals. (Wright, 2016)

Yoghurt is considered a nutrient dense food, but any added ingredients and production methods will dictate the final nutritional content. Being
made from milk, yoghurt is typically a good source of high-quality protein and contains a highly bioavailable source of calcium. It can also be a source of iodine, phosphorus and potassium, as well as ascorbic acid (B2) and vitamin B12. However, some yoghurt products are fortified with vitamin D, or may be enriched with extracalcium qualifying them for bone health claims (Ruxton; Phillips, 2015).

The health promoting properties of live lactic acid bacteria in yoghurt include protection against gastrointestinal upsets, enhancing digestion of lactose by male digesters, decreasing risk of cancer, lowering blood cholesterol and improving immune response and helping the body to assimilate protein (Owiah et al., 2017). Probiotics such as Bifidobacterium are commonly incorporated into yogurts worldwide. Positive health effects of bifidobacteria on human were well reported (Horackova et al., 2015). However, in Sudan probiotic soymilk yoghurt was not reported.

Objectives

1. To determine proximate composition of soya bean.
2. To evaluate the growth of Bifidobacterium longum BB536 during fermentation of soymilk yoghurt and at refrigeration storage.
3. To determine the physicochemical characteristic and nutritional composition of probiotic soymilk yoghurt.
4. To assess sensory properties of different processed yoghurts.
2.1 Milk

A mixture of Fat, Protein, Carbohydrate, Minerals and vitamins the fat and protein exists as a colloidal suspension in a mixture of water which contains water soluble carbohydrate and minerals(Wright,2016). Milk is the primary source of nutrients infants of mammals and defined as the secretion from mammary glands of mammals). (Gohansson, 2016).

2.2 Milk composition

The chemical composition of milk can be influenced by several factors such as animal species and genetic environmental on average, Bovine milk is composed of 87% water, 4% to 5% lactose, 3% protein, 3% to 4% fat,0.8% minerals, and 0.1% vitamins .(Mourad;Bettache,2014).

2.3 Milk constituents

2.3.1 Water

Water is the nutrient required in the highest quantity and milk contains a lot of water (87.6%). This amount of water controlled by the amount of lactose synthesized by the secretory cells of the mammary gland(Mourad; Bettache, 2014).

2.3.2 Protein

Milk proteins (casein and whey protein) are important protein source for human nutrition(Guo; Wang, 2016).
Milk proteins are heterogeneous mixture of proteins, and constitute approximately of 3.0%-3.5% of bovine milk. About 80% bovine milk proteins are called casein, which precipitates at PH 4.6.

The protein remains that soluble in milk serum at PH 4.6 are called whey protein. In general, casein and whey proteins have very different properties. Casein is mostly a random coil with a high proline content, phosphorylated for calcium pending, and exists in large casein micelles. Whey protein has ordered secondary structures with a low proline content, is non-phosphorylated, and has small soluble proteins. In addition, casein is sensitive to acid and is stable when heated, while whey protein is stable in acid and is sensitive to heat (Guo; Wang, 2016).

The proteins in milk are of great quality, they contain all the essential amino acids, and elements that our bodies cannot produce. It is important to remember that proteins are the building blocks of all living tissue. Milk proteins have roughly the same composition as the egg protein, except for the amounts of methionine and cystine, which are significantly lower. The sulfur amino acids are the limiting factors in milk. Casein and, even more, the complex milk protein contain good proportion of all amino acids essential for growth and maintenance (Mourad; Bettache, 2014).

The denomination crude protein (CP) includes protein (TP) and non-protein nitrogen (including urea). The protein content is an important feature of the milk. The TP determines the market value of milk, the higher the TP value is compared to a reference, the more money the producer will get. In fact, the more TP, the higher the yield of cheese making. Milk proteins represent 95% of crude protein, but the remaining 5% is: free amino acids, small peptides and non-protein nitrogen. Protein comprise either only amino acid (β-lactoglobulin, α-lactalbumin) or
amino acid and phosphoric acid (α and β casein) with a carbohydrate portion (sometimes even k casein). The base of the precipitation at pH 4.6 (20°C) or under the action of the rennet separates two components: caseins (α, β, γ and k) and the soluble protein or whey protein (Mourad; Bettache, 2014).

2.3.3 Casein

The four major caseins that exist naturally in milk are αs1 caseins; αs2, B and k. Caseins are distinguished by their low solubility at pH 4.6 and are differentiated on the basis of the distribution of exchange and sensitivity to precipitate (Mourad; Bettache, 2014).

2.3.3.1. Casein K

Among the most studied casein is casein k (k-CN), probably because of its importance in the stability of the micelle and its role in dairy processing. The k-CN is also the only casein having carbohydrate residues in its constitution. Caseins (α, β and κ) in the presence of calcium phosphate, form stable casein micelles (colloidal phase), which are balanced with the soluble phase of milk. It is possible to adjust the balance in terms of temperature, pH and the addition of salts. So long as the lactic acid bacteria convert lactose into lactic acid, it lowers the pH of the milk thereby decalcifying the casein micelles. There is another way to destabilize casein micelles and it is by using an enzyme such as cytosine (Mourad; Bettache, 2014).

Due to form α-helix and β-sheet, which are essential factors for secondary protein structures? About 85% to 90% of casein in bovine milk exists in colloidal form, known as micelles, which are porous, spherical aggregates with diameters ranging from 50 to 600 nm. Various models of casein structure have been suggested. It has been suggested that the integrity of
the micelle is attributed to the hydrophobic interactions between casein molecules, between caseins and calcium phosphate nano-clusters, or between polymeric casein aggregates and casein-calcium phosphate aggregates. The structure of the casein micelle is still elusive, but the “hairy” layers of K-casein on the surface of the micellar structure are generally accepted. The casein micelle is extremely stable in heat treatment, due to the K-casein layers. K-casein is the only glycosylated casein with oligosaccharides attached to threonine residues in the C-terminal, and the only calcium insensitive casein (Guo; Wang, 2016). The C-terminal, due to the increased hydrophilicity, is the “hairy” structure that extends into the serum solution, which contributes a stabilizing force. During the production of cheese or rennet casein, chymosin is added to cut off the -casein tail, and the casein collapses to form a weak gel due to the loss of the inter-micellar repulsion force. That is why sweet whey protein (the whey produced from cheese making) contains a significant amount of Glycomacropeptide (GMP), which is one third of K-casein and is present in whey protein products. The casein micelle is made up of 94% casein protein and 6% CCP. During acidification, such as using acid casein, in yoghurt making, or in the natural milk-souring process, CCP is dissolved when the pH drops and causes a gradual dissolution of micellar integrity, and then casein starts to aggregate or precipitate. The casein micelle is a very complex polymer aggregate, and interacts via hydrophobic and electrostatic interactions and calcium bridging. A typical casein micelle contains about 10,000 casein molecules. In the past, bovine casein molecule was usually called random coil protein because of the high content of proline residues, which prevents the formation of -helix or -sheet structures. Casein has extreme heat stability and shows no evidence of denaturation to a more disordered structure. The term “theomorphic” was also suggested for this type of protein. Caseins, considered
theomorphic proteins, have been adopted by many researchers. According to de Kruif and Holt’s, (2003) definition, a rhomorphic protein is “one with an open conformation and therefore has a considerable degree of side chain, and possibly also backbone, conformational flexibility (Guo; Wang, 2016).

2.3.4 Whey Proteins

Other milk proteins are present in the whey serum and whey proteins are defined as soluble proteins in the whey after precipitation of caseins at pH 4.6 and at 20°. Serum proteins include a first protein fraction (80%) consisted of β-lactoglobulin (β-LG), β-lactalbumin, bovine serum albumin (BSA) and immunoglobulin. A second non-protein fraction (20%) is composed of protease, peptone and nitrogen compound. Whey is the liquid that remains after milk is curdled or strained. In the dairy industry, whey refers to the co-products during the process of cheese, casein, or yogurt manufacturing. Cheese whey is currently the most abundant commercially available whey product. Whey proteins are a group of soluble proteins that are present in the milk serum phase at a pH of 4.6 (Mourad; Bettache, 2014).

Beta-Lg is the dominant protein in whey, which accounts for about 50% of total whey proteins. The molecule is comprised of 15% alfa-helix, 50% beta-sheet, and 15%–20% reverse turn. It has a very organized secondary structure, which is described as a cup or calyxshaped three-dimensional structure. Each monomer has one thiol group, and two disulphide bonds. The presence of the thiol group and the disulphide bond gives beta-Lg an excellent gelation property via a thiol-disulphide interchange under heat treatment. beta-Lg can exist in the form of a monomer, dimer, or octamer
via the disulphide bonds, depending on pH, temperature, and ion strength. It exists as a dimer in neutral pH aqueous solutions (Guo; Wang, 2016).

2.3.5 Fat

In milk, fat is the main source of energy. Goat and cow milk are low in polyunsaturated fatty acids that are necessary for human metabolism. Fat is present in milk in the form of an emulsion of fat cells; the concentration of the fat content of milk can be found in small cells suspended in water which varies considerably by race and composition of feed. The unsaturated fatty acids are lipid molecules containing at least one double bond, according to the structure of this or of these double bonds. It is possible to distinguish the cis fatty acids and trans fatty acids. Most unsaturated fats in our diet are in the cis form, and a lower proportion is in the trans form. The fat content of goat milk is slightly greater than that of cow milk, in both cases; triglycerides represent more than 95% of total lipids. Phospholipids comprise 30 to 40 mg/100 ml of cow milk which contains 8 to 10 mg of lipid, cholesterol is from 10 to 20 mg per 100 ml, most of which is in the free form (Mourad; Bettache, 2014). During the homogenization of milk, the fat globule increases in number and considerably decreases in diameter (less than 1 micron). Therefore, the contacts are increases about 20 times. This change prevents fat from rising (in the long-life milk) and promotes digestion. The lipid composition of milk includes two major groups: simple lipids (triglycerides) and complex lipids (phospholipids). The milk fat has a nutritional role by energy intake, 9 kcal/g as dietary lipids (Mourad; Bettache, 2014).

Finally, milk fat provides essential vitamins to the body: vitamins A and D above; Vitamin A is essential to the epithelia, hence its role in reproduction, vision, while Vitamin D is essential in the binding of
calcium and bone growth. The fat has a very important sensory role during tasting; it gives a creamy texture appreciated, smooth, and velvety, fondant, etc. On the other hand, many flavors are associated with fat; it intervenes in intensity, balance, after taste of these aromas. The reduction or elimination of lipids from dairy products will therefore require technological improvements, to keep the finished products familiar organoleptic qualities. Fat plays an important role in determining the flavor and texture of the finished product. Note that the fat globules are separable by microfiltration and fractional crystallization, making it possible to obtain products with a specific character. Chemical changes that must be taken into account in the choice of process technology and the use of additives such as antioxidants (Mourad; Bettache, 2014).

2.3.6 Carbohydrate

Lactose is the main carbohydrate of milk. It is formed by the union of one molecule of D-galactose (engaged by its semiacetylfunction) and one molecule of D-glucose (committed by its hydroxyl 4 position). It has a β-galactoside 1,4 bond (which is hydrolyzed by a β-galactosidase) and is a 4-Dglucopyranosyl-β-D-galactopyranose. Although lactose is a sugar, it does not have a sweet flavor (Mourad; Bettache, 2014). Its concentration varies slightly in milk (4.5 to 5.2 g / 100 g) contrary to the concentration of fat, that of lactose cannot be easily modified by feeding and true step of a dairy race to another. It is used as substrate during the fermentation of milk by lactic acid bacteria, differing in the fermented productssuch as yoghurt and cheese. It plays a role in fermented milk production. The amount of lactic acid produced by lactic acid bacteria in a fermented milk product depends not only on the bacterium itself (the bacterial strain moreless active) and operating
parameters, but also on the available amount of lactose bacteria (Mourad; Bettache, 2014).

Lactose mainly provides the infant mammal energy but also have other functionssuch as giving milk the sweet flavor. For the blood to be able to take up lactose it must first be hydrolyzed by the enzyme lactase to glucose and galactose.

Individuals whom metabolize lactose poorly due to a significant reduction in lactase activity are called lactose mal-absorbers. This condition can eventually lead to lactose intolerance (Guo; Wang, 2016).

### 2.3.7 Vitamins and minerals

Milk contains both fat-soluble and some water soluble vitamins. Vitamin A can naturally be found in the fat component of whole milk. It is not unusual to fortify milk with vitamin A prior to sale since the separation process of milk leads to small amounts of vitamin A in the low fat milk. Vitamin D is also naturally present in whole milk though in small amounts and therefore it is commonly fortified as well (Majid, 2016).

Also vitamins B6, B12, C, K, E, thiamin, niacin, riboflavin, biotin, and pantothenic acid are all present in milk (Aradaib, 2017). Calcium and phosphorus are the main minerals present in milk.

Calcium can be found combined with the protein casein as calcium caseinate, with phosphorus as calcium phosphate and as calcium citrate. Furthermore, magnesium, potassium, sodium and sulfur are other minerals present in milk (Guo; Wang, 2016).

### 2.3.8 Enzymes

Enzymes are specific globular proteins produced by living cells.
Each enzyme has its isoelectric point and is susceptible to various denaturing agents such as pH change temperature, ionic strength, and organic solvent (Mourad; Bettache, 2014).

2.4 Legumes

Grain legumes play an important nutritional role in the diet of millions of people in the developing countries and are thus sometimes referred to as the poor man's meat. Since legumes are vital sources of protein, calcium, iron, phosphorus and other minerals, they from a significant part of the diet of vegetarians since the other food items they consume don't contain much protein. (Mohammed, 2017).

Due to the diverse roles played by grain legume crops in farming system and nutritional security, the research on legume crops will have significant impacts on nutritional security and soil fertility, especially in the developing countries. The recent rise in prices has led to an increase in demand for legumes worldwide through both income and population growth (Mohammed, 2017).

Moreover, the grain legume crops have potential health benefits, which include reducing cardiovascular, diabetic and cancer risk (Mohammed, 2017).

2.4.1 Soybean

Soybean scientific name (Glycine max) local name (sudan 2) common name: large bean, yellow bean, edamame (Japan), golden bean, miracle bean, Soyabean belong to the family leguminosae. Seed almost spherical in shape are usually light yellow, but some varieties are black, brown or green. The first historical places of soybean as a food crop in north-eastern china, soybean used in various country Burma, India, Indonesia, Japan,
Korea, Malaysia, Philippines, Nepal, Thailand, and Vietnam, England, USA. (CNUCED, 2016)

Soya bean seeds planted 1 ½ deep best grows in temperate, tropical and subtropical regions. Growing varies from 50 to 200 days, grown throughout the year in the tropics and sub tropics, if water is available. It requires well-drained and fertile soil, 400 to 500 mm precipitation in a season for good crop. at the time of germination High moisture it is requirement, In the stage flowering and pod forming dry weather is essential for ripening. (CNUCED, 2016)

2.4.2 Soybean production

World soyabean production is dominated by three countries: the US, Brazil and Argentina.

**Top 10 soya bean producer in 2013**

<table>
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<tr>
<th>Production (tones)</th>
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<tbody>
<tr>
<td>United states of America</td>
<td>89,483,000</td>
</tr>
<tr>
<td>Brazil</td>
<td>81,724,477</td>
</tr>
<tr>
<td>Argentina</td>
<td>49,306,201</td>
</tr>
<tr>
<td>China</td>
<td>11,950,500</td>
</tr>
<tr>
<td>China, mainland</td>
<td>11,948,000</td>
</tr>
<tr>
<td>Paraguay</td>
<td>9,086,000</td>
</tr>
<tr>
<td>Canada</td>
<td>5,198,400</td>
</tr>
<tr>
<td>Uruguay</td>
<td>3,200,000</td>
</tr>
<tr>
<td>Ukraine</td>
<td>2,774,300</td>
</tr>
<tr>
<td>Bolivia(plurinational state of)</td>
<td>2,347,282 (CNUCED, 2016)</td>
</tr>
</tbody>
</table>
In Nepal, India, and Bhutan. Soybean was probably introduced into India from China through the Himalayas several centuries ago and some believe that soybeans were also brought via Myanmar by traders from Indonesia (Jyoti, 2015). First introduced of soya bean to sub-saharan Africa by Chinese traters in 19th century, in 1903 the first record of cultivation as an economic crop in South Africa. Soya bean first cultivated in Tanzania in 1907 and Malawi in 1909. Introduced soya bean in Nigeria in 1908, citivated as an export crop in small area in benue state. First introduced soybean in Sudan in 1910. Further introductions in the country in 1912 and in 1949, planted soya bean in southwest Sudan to prevent severe malnutrition. Other countries including in SSA Zimbabwe, Malawi, Ghana, Sudan, and Ethiopia (Dalia, and etal, 2018)

2.4.3 Nutritive value

Soyabean have an exceptional nutritional and functional food profile. soy-food are considered to be nutirious and healthy based on their nutrient composition. (UNUCED, 2016) It is an excellent source of protein and oil good quality contains about (43%) protein, (21%) carbohydrates, (5%) minerals, (8%) moisture, (20%) fat, (4%) fiber (Deshmukh, 2016)

2.5 Composition of soybean

2.5.1 Soybean oil

Soy oil is intensively consumed as a vegetable oil worldwide. It is used as cooking oil in households but its main use is as key fat ingredient in industrial food manufacturing. Diets with potentially negative health implications are those where saturated fatty acids and/or trans fatty acids
are in excess of certain desirable levels, while diets including oils/fats rich in mono or poly-unsaturated fatty acids tend to be considered as more healthy. Nutritional properties of soy foods and soy oil are the following. Soybeans are high in fat. Most legumes (except peanuts) contain between 2 to 14 % fat, whereas soybean contains 19 % fat (CNUCED, 2016).

2.5.2 Fibre

Fibers help to facilitate the digestive process and put far less strain on the gastrointestinal system. Soluble and insoluble carbohydrates, including dietary fiber, make around 30% of the soybean. The primary soluble carbohydrates in the soybean are the sugars stachyose, raffinose and sucrose. A serving of soybeans provides approximately 8 grams of dietary fibre. However, processed soy food fibre contents decrease significantly. Tofu and soymilk contain very little amount of fibre, while soy foods that utilize the whole bean such as tempeh, soy flour and textured soy protein have high level of fiber (CNUCED, 2016).

2.5.3 Protein

The soybean is a valuable legume because it does provide all of the essential amino acids for humans; however it is relatively low in the sulfur containing amino acids, cysteine and methionine. It is one of the few legumes that can be consumed as a complete protein. The soybean is comprised of approximately 37-42% protein (Sean; Laurie; and Jyostna, 2015).

The level is higher than in other beans, and therefore soybeans protein is equivalent to animal protein in quality. They are highly digestible and are comparable to beef, milk, fish, and egg protein in terms of protein quality. Based on the internationally recognized Protein Digestibility Corrected Amino Acid Score (soybeans protein isolate receives a rating of 1, which
is the highest possible score. It makes soybeans equivalent to animal proteins in quality and higher than other plant proteins foods. Soy foods, if used correctly, contribute significantly toward meeting protein needs (CNUCED, 2016)

2.5.4 Calcium

Soy foods are a good source of calcium in comparison to the commonly used legumes. However, processing affects the calcium content of soyfoods considerably. Although soyfoods are high in both oxalates and phytates that inhibit calcium absorption, the calcium from soyfoods is well absorbed and has an absorption rate equal to that of milk (CNUCED, 2016).

2.5.5 Iron

Soybeans are rich in iron. However, both phytate and soy protein reduce iron absorption which leads to a poor absorption of soyfoods. Iron could be better absorbed from fermented soyfoods such as tempeh and miso (CNUCED, 2016).

2.5.6 Other nutrients

The major mineral components of soybeans are magnesium, potassium, sodium, calcium, sulfur and phosphorus (cadmium was below detectable range, zinc, and cooper(AJOPACS, 2017). Mineral content can vary widely due to the type of soil and growing conditions for the soybean. Soybean is a good source of enriched Calcium and Vitamin B12. Tocopherols are an important constituent of soy oil, due both to the vitamin E supplied for human nutrition and their antioxidant properties (CNUCED, 2016)
2.6 Soya product

Today soymilk, tofu, yuba, and the fermented products natto, miso, tempeh and soy sauce continue to be important foods in Asian countries. Soy processors convert soybeans into products made from whole soybeans, such as soy nuts, tofu, tempeh, miso, natto, soy sauce, some soy flours, soybean butter and soymilk. Other soybeans destined for more traditional food and technical products are graded, cleaned, dried, and cracked to remove the hull. Soybean hulls are further processed for animal feed or fiber additives for breads, cereals, and snacks (Deshmukh, 2016).

**Tofu, Dan fu (Vietnamese), Teou fu or Tou fu ho (Chinese) or bean curd:** Soybeans are washed and soaked overnight in water, and then ground in a stone mill into a slurry. Salting the salt until salt content of pehtze reaches about 16%, which takes 6–12 days and then Ripening (IGO FM, 2001)

**Soya sprouts:** Germinated soybeans in dark yellow cotyledons with white sprouts. **Soya film, Yuba:** Creamy, yellowish protein-lipid film formed from the surface of boiling soya drink (in sticks, or flakes). **Vegetable soybean, Edamame:** Immature green soybeans. They are harvested when the beans are still green and sweet tasting and can be served as a snack or main vegetable dish after boiling in slightly salted water. Edamame is high in protein and fiber. **Roasted soybeans:** Dry roasted soybeans, seasoned or non-seasoned (CNUCED, 2016).

**Miso** is produced in the form of paste produced by fermentation with help of lactic acid and yeast commonly in households of Japan. The local people developed taste but different range of Miso exists can be divided into tow categories: 1. standard Miso made from soy beans, water, salt, and rice or barley, there were special fortified nutritional Miso contains
calcium, vitamin B1, B2, or B1; 2. low salt Miso which is used as therapeutic food in hospital (Simarn, 2017). **Tempeh:** Cooked and dehulled soybeans fermented with a fungus named *Rhizopus oligosporus*. The soft beans are bound by white mycelia, cake-like, nutty flavor. Tempeh is one of the most popular fermented foods in Indonesia, New Guinea and Sumatra. Because of its meat-like texture and mushroom-like flavor, tempeh is well suited to Western tastes. It is becoming a popular food for vegetarians in the United States and other parts of the world. **Shoyo, Soya sauce, Soysauce:** Extracted from a fermented mixture of soybeans and wheat (CNUCED, 2016). **Natto:** Natto, a traditional fermented soybean product in Japan, is produced by fermenting steamed soybeans with the bacterium Bacillus subtilis var. natto and by aging it in cold/freezing condition after fermenting. Natto contains a variety of nutrients such as high-quality protein, vitamins, minerals, and dietary fiber in a balanced manner. It is widely recognized as a healthy food and traded and consumed mainly in Asian regions (CAC, 2016).

### 2.7 Utilization of soya milk:

Non-fermented soy foods, Soymilk has been produced traditionally in China, and in East Asia, even it was never a predominant element in the popular diet. The concept of a milk-like food made with soybeans was introduced to Europe in the beginning of the century (UNUCED, 2016). Soymilk beverage worldwide is credited to health benefits e.g. low cholesterol and lactose, its ability to reduce bone loss and menopausal symptoms, prevention and reduction of heart diseases and certain cancers. As this drink is cholesterol free and low in energy, it could enhance health benefits in terms of reducing body weight and blood lipids. With its unique nutty flavor and rich nutrition, soymilk can be used as supplementary way of dairy milk. It is available as a plain, unflavored
beverage or in a variety of flavored beverage including chocolate, vanilla and almond (Deepika, and etal, 2017).

**Soymilk:** Soymilk is an aqueous, white, creamy extract produced from soybeans which is similar to cow milk in appearance and consistency. It is a highly nutritious which contains protein, fat, carbohydrates vitamins and minerals (Deepika, and etal, 2017).

### 2.7.1 Preparation of soy milk

Soybean sorted and cleaned to remove stones and damaged, deformed seeds. Then the dry soybean was washed and soaked in water (500g in 1 Liter) for 12 hours. It was then rinsed and blanched in 1.25% NaHCO3 for 30 minutes. The rehydrated soybean was washed, manually dehulled and rinsed. The soybean seeds were ground in blender and expressed in the ratio of 3:1 (water to beans on a weight basis) to remove the okra. The obtained milk is then formulated by adding anti-oxidants and preservatives. The milk was then pasteurized at the temperature 71°C for 15 seconds and subsequently bottled and stored at ambient and refrigeration temperature(Deepika, and etal, 2017).

### 2.8 Soymilk Yoghurt

**Yoghurt:** is one of the oldest fermented milk products known in human history. It is produced by inoculating concentrated milk with a yoghurt starter culture consisting of a mixture of homofermentative lactic acid bacteria, *Strephococcus thermophilus* and *Lactobacillusbulgaricus* at 45°C until the pH of about 4.0 is attained and cooled rapidly to about 4°C. Yoghurt is consumed for its refreshing and appealing flavor(IJON, 2017).

Wide spread acceptability of soymilk by consumers is been hindered by the intrinsic beany flavor associated with soybean .Thus in a bid to win consumers appeal, composite soymilk yoghurt was innovatively
formulated and produced to meet the need of both the young ones who do not bother about cholesterol as well as the increasing number of lactose intolerance children and the adult consumers who want a healthy soymilk yoghurt that has a low cholesterol level than cow milk yogurt, leading to a healthy active life and good taste with smooth texture, and reducing the cost of yoghurt in the market (IJON, 2017). Soymilk yoghurt has been studied extensively Fermented soy milk products may be beneficial from an economic and nutritional point of view since they can be prepared with higher protein levels at a comparable or lower cost than regular fermented milk products. Soy proteins have a favorable amino acid balance meeting the essential amino acid requirements except for methionine. The research of a number of authors has indicated a lot of advantages of soymilk products for the nutrition of children and adults suffering from allergies, diabetes, cancer, heart and renal diseases. Soymilk products and soymilk yoghurt can successfully replace the fermented milk products from cow’s milk (Zapryana, 2015).

Yoghurt products have high viscosity of the finished product and generally set in weak gel. To enhance gel formation and to increase the viscosity of the finished product a high solid content is desirable. In most cases, concentrated milk or non-fat dry milk is added to increase the solid content (Oyeniyi; Aworh; Olaniyan, 2014).

Processing and fermentation of soy products reduce the isoflavone levels significantly (Sean; Laurie; and Jyostna, 2015).

2.8.1 Nutritive value

Yoghurt is considered a nutrient dense food, but any added ingredients and production methods will dictate the final nutritional content. Being made from milk, yoghurt is typically a good source of high-quality protein and contains a highly bioavailable source of calcium. It can also be a
source of iodine, phosphorus and potassium, as well as riboflavin (B2) and vitamin B12. Although not naturally a source, some yoghurt products are fortified with vitamin D, or may be enriched with extracalcium qualifying them for bone health claims. The fat content of yoghurt varies widely, ranging from ‘fat free’ varieties with less than 0.5% fat, through to low-fat yoghurts containing less than three percent fat and up to 10 percent for some Greek style types (Carrie, Frankie, 2015).

The health promoting properties of live lactic acid bacteria in yoghurt include protection against gastrointestinal upsets, enhancing digestion of lactose by maldigesters, decreasing risk of cancer, lowering blood cholesterol and improving immune response and helping the body to assimilate protein (Sampson, and et al, 2017)

2.8.2 Manufacturing of yoghurt

The fundamental basis of yoghurt manufacturing is the acid coagulation of milk proteins, i.e. mainly caseins, by the activity of starter cultures. The milk coagulation results in a three-dimensional gel network capable to capture the liquid serum phase. Based on the physical characteristics, three common types of yoghurt are available in the daily marketplace: (i) set-yoghurt, (ii) stirred-yoghurt and (iii) drinking yoghurt. Set-yoghurt is usually fermented in package; and has a firm gel-like texture and natural flavor associated with its more traditional image. Stirred-yoghurt is sheared after fermentation, which produces a semi-solid pourable product, and then stabilizers, fruit mixtures as well as other ingredients are commonly added. Drinking yoghurt is produced by mixing an ordinary yoghurt with water and/or fruit concentrate, resulting in a low viscosity and drinkable characteristics of the product. Although manufacturing stages of the three types of yoghurt have much in common, the
acidification rate greatly differs between set-yoghurt and stirred-yoghurt due to the difference in inoculums size and incubation temperature. This condition reflects substantial changes on the rheological and organoleptic quality of the fermented products (Sarn, 2014).

2.8.3 Homogenization

Immediately after pasteurization, the milk was homogenized. The order of addition of various ingredients was determined by their temperature and solubility. The mix was then cooled rapidly in cold-water bath to 42-45°C, after having obtained a homogenous mix (Oyeniyi; Aworh; Olaniyan, 2014).

2.8.4 Fermentation Process

After homogenized soy milk were inoculated with 2.5% commercial yoghurt starter 50:50 mixture of Lactobacillus bulgaricus and Streptococcus thermophilus. The mixture was stirred well and retained at a temperature of 41°C for a period of 3 hours. At the end of the incubation period, the output was yoghurt (Amjad, 2017).

2.8.5 Soy milk Yoghurt ingredient

Soy milk

Cow milk

Skim milk

Flavoring materials

Bifidobacteria
2.8.5.1 Flavoring materials

Banana, orange and strawberry flavor essence yogurts prepared and fruit flavor essences were obtained from Two different formulations used for production fruit flavored yogurts with each fruit flavor essences that first formulation manufactured with 0.5 % (Amjad, 2017).

2.8.5.2 Bifidobacteria

**Bifidobacterium Scientific Classification**

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phylum</td>
<td>Actinobacteria</td>
</tr>
<tr>
<td>Class</td>
<td>Actinobacteria</td>
</tr>
<tr>
<td>Subclass</td>
<td>Actinobacteridae</td>
</tr>
<tr>
<td>Order</td>
<td>Bifidobacteriales</td>
</tr>
<tr>
<td>Family</td>
<td>Bifidobacteriaceae</td>
</tr>
</tbody>
</table>

(Rawia; salma; and samah, 2015)

Bifidobacteria are Gram-positive branched rod-shaped bacteria belonging to the phylum of *Actinobacteria*. They are non-spore forming non-motile anaerobic microorganisms producing acid but not gas from various carbohydrates. The species of this genus was first discovered in 1899 by Tissier and was referred to as *Bacillus bifidus*, but later was classified as Bifidobacterium *bifidum*. Thirty-two species of *Bifidobacterium* have been discovered so far, including *B. tsurumiense* and *B. mongoliense*, identified in 2008 and 2009, respectively. The species were isolated from human intestines, oral cavities, blood, food, animal and insect intestines, and sewage. The gastrointestinal tract or infant stool samples are rich in
facultative anaerobic Proteobacteria. Later on, when oxygen levels decrease, anaerobic species belonging to Bacteroides, Clostridium and Bifidobacterium colonize the gut, the bacterial composition in the gut becomes similar to that of adults (Avershina, 2011).

B. bifidum strain was already present in your intestines, protecting from pathogenic microbial groups germs. In fact, it made up nearly 95% of your intestinal bacteria attaches to intestinal lining and helps digest sugar. It also keeps germs and toxins away from blood while it stimulates your immune system (GHC, 2015).

Bifidobacterium spp. normally inhabits the human gastrointestinal tract and is one of the main probiotic cultures. As an example of the positive health effects of bifidobacteria can be their ability to bio-activate some substances which can increase their impact on human body. Isoflavones belong to a group phytoestrogens with the highest level of estrogenic activity. In plants, they occur as free aglycones or are very commonly bonded with glycosides or their malonyl and acetyl conjugates. Isoflavone glycosides derived from soymilk require bacterial-induced hydrolysis in order to be transformed into a bioavailable aglycone form discovered. Isoflavone aglycones are absorbed faster and in higher amounts than their respective β-glucoside forms.

The reduction of the beany flavour of soymilk, which improves the sensory quality of the final product could be also connected with fermentation. Bifido bacteria strains, specifically, are able to metabolise n-hexanal and pentanal in soymilk the former is responsible for the undesirable flavor. Furthermore, fermentation also reduces the content of soy galactooligosaccharides: indigestible carbohydrates, which can in higher amounts cause undesirable flatulence (Horackova et al; 2015).
CHAPTER THREE

MATERIALS AND METHODS

3. Materials

3.1. Raw materials

Soya bean seed samples were obtained from Agricultures Research Corporation headquarter (wad madani, Aljazeera State, Sudan). Fresh cow milk was obtained from department of Animal Production, College of Agricultural Studies, Sudan University of Science and Technology. Chemicals analytical grade were used.

3.2. Methods

3.2.1. Production of soymilk

300gsoybean was washed by tap water and then soaked in distilled water for 12 hours at room temperature. The hulls were removed manually. The de-hulled bean were blended with 900 ml distilled water at high speed for 10 minutes, then filtration. The obtained soymilk was pasteurization at 85 °C for 15 minutes then used directly.
Flow diagram 1: Soymilk production

1. Cleaned whole soybean
2. Soaked in distilled water for 12h
3. De-hulling
4. Blended with water for 10 min
5. Filtration of soymilk
6. Soya milk
3.2.2. Preparation of fermentation inoculums

*B. longumBB536* was maintained at -20 °C in 20% glycerol solution. Stock culture was prepared by activation of the strain in skim milk, incubate an aerobically at 37 °C for 24h. The obtained culture was reactivated again under the same conditions to prepare enough stock for the experiment. The working culture was prepared by twice successive transformation in 10% sterilized skim milk (121°C for 15 min) and incubation at 37 °C for 24h.

3.2.3. Processing of yoghurt

Soy milk and cow milk pasteurized, cooled and inoculated with a 10% active culture of *B. longumBB536* followed by incubation at 37 °C for 3 h.
Flow diagram 2: Production of soy milk yoghurt

1. Soy milk pasteurize (85°C, 15 min)
2. Homogenize
3. Cooled to 37°C
4. Added starter culture (*Bifidobacterium*)
5. Incubate at 37°C for 3 hours
6. Cooled to 4°C
3.3. Determination Chemical composition

3.3.1. Determination of moisture content

Moisture was determined according to the modified method of AOAC (1990). 5 grams of the sample was weighted in a sensitive balance, after weighting the dishes were transferred to an oven (Kat-NR. 2851, Electrohelios, Sweden) at 105 ± 0.1°C for 6 hours. Afterwards, the dish with sample was transferred to desiccators and allowed to cool at room temperature before reweighting. Moisture content was calculated according to the following formula:

\[
\text{Moisture content (\%) } = \frac{M_2 - M_3}{M_2 - M_1} \times 100
\]

Where:

\( M_1 \) = mass of dish + cover.

\( M_2 \) = mass of dish + cover + sample before drying.

\( M_3 \) = mass of dish + cover + sample after drying.

3.3.2. Determination of fat content

Fat content was determined according to the official method of AOAC (1990). A sample of 5g was weighed into an extraction thimble and covered with cotton, and then extracted with hexane. The thimble containing the sample and a pre-dried weight extraction flask containing about 100 ml hexane was attached to the extraction unit. The extraction process was conducted for 16h. At the end of the extraction period, the flask was disconnected from the unit and the solvent was evaporated.
Later, the flask with the remaining crude hexane extracted was put in an oven, cooled to room temperature reweigh and the dried extract was registered as fat content.

Crude fat content (%) =

$$\frac{W2 - W1}{\text{Sample of weight}} \times 100$$

Where: $w1=$ The weight of the empty extraction flask.

$W2=$ The weight of the extraction flask after the extraction process.

### 3.3.3. Determination of protein content

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

**Digestion:** two gram of the different yoghurt were weighed in a crucible and transferred to a digestion flask with two tablets catalyst (mercury). 25 ml of concentrated sulphuric acid were added to the samples, the flask was placed on the digestion apparatus, heated until the mixture was colour less. Then the flasks were allowed to cool.

**Distillation:** 25 ml of boric acid and three drop of bromocresol green+ methyl red indicator were added to each receiving flask. The digested samples were transferred from the digestion flask to volumetric flask and the volume was completed to 100 ml by distilled water. The receiving flask was placed on the distillation rack with the tip of the condenser extended below the surface of the acid. Immediately 5 ml of the diluted samples 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 flasks were 7 ml, then the flask were removed from the distillatory.

**Titration:** The samples in the receiving flask were titrated against 0.1 N HCL. The colour was change from green to purple. The nitrogen content was calculated as follows:

\[
N\% = \frac{m \times \text{Normality of HCL}(0.1) \times 0.014 \times 100}{\text{Sample weight}}
\]

Protein (%) = (N %) × 6.25

Where N = Nitrogen content.

0.014=molecular weight of nitrogen/1000

**3.3.4. Determination Ash content**

The ash content of samples was determined according to the AOAC (1990) method. A 2g of the deferent yoghurt were weighed into a clean dry porcelain crucible and placed in muffle furnace (model Tipoforon Z A No 18203 Get Ran 1002) at 600°C for 6 hours. The Crucible was transferred to desiccators, cooled to room temperature and weighed .The ash content was calculated as follows:

\[
\text{Ash content (%) } = \frac{W_1 - W_2}{\text{Weight of sample}} \times 100
\]

Where:

W1 = Weight of crucible with ash.

W2 = Weight of empty crucible.

**3.3.5. Calculation of carbohydrates**

Carbohydrates were calculated by difference according to the following:
Total carbohydrates = 100% - [Moisture (%) + Protein (%) + Fat (%) and Ash (%)].

3.3.6. The storage of yoghurt

Yoghurts were held at refrigerator for a period of 2 weeks. During the storage period, viable counts of *B. longum BB536*, pH, Total carbohydrate and moisture were determined. Analysis for samples carried out at initial (0 days), after 1 week and after 2 weeks.

3.3.6.1. Enumeration of viable cell

MRS medium was used to enumerate *B. longum BB536* of different yoghurts using the plate count technique. 1ml yoghurt was diluted in peptone water, followed by plating on Rogosa agar (MRS) supplement with 0.05% L-cystiene. The plates were incubated aerobically at 37 °C for 48 h. The growth was calculated as Colony Forming Unit per ml (CFU/ml).

3.3.6.2. Determination of total solids (TS)

Total solids (TS) of yoghurt were determined at room temperature using digital refractometer with degree Brix° scale 0-100 according to AOAC (1990).

3.3.6.3. Determination of pH value

The pH value of yoghurt was determined using a pH-meter (model HI 8521 microprocessor bench PH/MV/C° meter. Romania). Two standard buffer solution of pH 4.00 and 7.00 were used for calibration of the pH meter at room temperature. The pH meter was allowed to stabilize for one minute and then the pH of yoghurt samples was directly measured.
3.3.7. **Statistical analysis:**

One-way ANOVA were preformed to examine significant differences between normally distributed data of replicated measurement. Probability less than 0.05 was considered significant \((p \leq 0.05)\). All data were analyzed using vision 17MINITAB statistical soft ware for windows (2007).
CHAPTER FOUR
RESULTS AND DISCUSSION

4.1 Proximate composition of raw materials

From the result in Table (4.1), soymilk contained 11 times protein, six times fat, four times ash and seven times carbohydrate higher than that of fresh cow milk. Overall, an average, Bovine milk is composed of 87% water, 4% to 5% lactose, 3% protein, 3% to 4% fat, 0.8% minerals, and 0.1% vitamins (Mourad; Bettache, 2014).

Table 4.1: Proximate composition of raw materials

<table>
<thead>
<tr>
<th>Parameter (%)</th>
<th>Fresh milk</th>
<th>Soya bean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>89.18</td>
<td>8.54</td>
</tr>
<tr>
<td>Protein</td>
<td>3.16</td>
<td>36.49</td>
</tr>
<tr>
<td>Fat</td>
<td>3.08</td>
<td>19.84</td>
</tr>
<tr>
<td>Ash</td>
<td>0.79</td>
<td>4.97</td>
</tr>
<tr>
<td>Total carbohydrate</td>
<td>3.80</td>
<td>20.86</td>
</tr>
</tbody>
</table>

Values are mean for replicate analysis
4.2. Chemical composition of different milks

Protein and fat of soymilk were higher than their levels in cow milk (Table 4.2). While ash and carbohydrates of cow milk were higher than in soymilk. Protein and fat contents of cow milk were slightly lower than the finding by Mohammed (2012) who reported 3.35% protein and 3.80% contents. Moisture content cow milk was 89.18%, the value was comparable with finding by Mohammed (2012) who reported 89.50%.

Soymilk was containing 6.6% protein and 3.57% fat, these result were higher than that reported by Oyeiniyi et.al. (2014), they stated value of 3.585%. Ash, moisture, total solids and pH contents were (Table ), this result was lower than those reported by Oyeiniyi et.al (2014) which were 0.69% ash, 89.74% moisture, 10.50% total solids and 7.00 pH.

Table 4. 2:Chemical composition of different milks

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Soymilk</th>
<th>cow milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture%</td>
<td>88.32</td>
<td>89.18</td>
</tr>
<tr>
<td>Protein%</td>
<td>6.6</td>
<td>3.16</td>
</tr>
<tr>
<td>Fat%</td>
<td>3.57</td>
<td>3.08</td>
</tr>
<tr>
<td>Ash%</td>
<td>0.45</td>
<td>0.79</td>
</tr>
<tr>
<td>Total solids%</td>
<td>9.48</td>
<td>10.83</td>
</tr>
<tr>
<td>Carbohydrate(as lactose)%</td>
<td>1.2</td>
<td>3.80</td>
</tr>
<tr>
<td>pH</td>
<td>6.71</td>
<td>6.58</td>
</tr>
</tbody>
</table>

Values are mean for replicate analysis
4.2. Physiochemical characteristics of different yoghurts

Results in Table (4.3) showed that cow milk yoghurt (A) was the highest TSS and pH as compared to yoghurt B and C. The TS was lower than that reported by IJON (2017) who stated value of 13.66% TS. PH content of all yoghurt was higher than 3.02% by as stated IJON (2017).

Table 4.3: Physiochemical characteristic of Soymilk yoghurt

<table>
<thead>
<tr>
<th>Sample</th>
<th>Moisture</th>
<th>TS</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>87.55±0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.54±0.64&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.81±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>B</td>
<td>87.67±0.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.53±0.65&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>4.71±0.04&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>C</td>
<td>87.26±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.54±0.65&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.80±0.12&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± SD; means carrying the same superscript letters in the same column are for significantly different P≤ 0.05 using Minitab.

A = 100% cow milk yoghurt.

B = 100% soy milk yoghurt.

C = 50% soymilk and 50% cow milk yoghurt.
4.3. Nutritional composition of different yoghurts

The result presented in Table (4.4) showed that soymilk yoghurt (B and C) contained higher level of protein, fat, ash and total carbohydrate than cow milk yoghurt (A). While IJON (2017) reported 4.2% protein, 1.88% fat, 0.91% ash and 7.58%.

Table 4.4: Nutritional composition of different yoghurt

<table>
<thead>
<tr>
<th>Sample</th>
<th>Moisture</th>
<th>Protein</th>
<th>Fat</th>
<th>Total Carbohydrate</th>
<th>Ash</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>87.55±0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.31±0.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.00±0.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.07±0.64&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.13±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>B</td>
<td>87.67±0.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.65±0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.51±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.85±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.14±0.09&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>C</td>
<td>87.26±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.81±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.25±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.25±0.49&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.37±0.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± SD; means carrying the same superscript letters in the same column are for significantly different P ≤ 0.05 using Minitab.

A = 100% cow milk yoghurt.

B = 100% soy milk yoghurt.

C = 50% soymilk and 50% cow milk yoghurt.
4.4. *Bifidobacterium longum* BB 536 in processed yoghurt

The levels of strain BB356 in all types of yoghurt (Table 4.5) were higher than 6 log CFU/ml fulfilling the requirement of probiotics foods.

4.4.1. Survival of *Bifidobacterium longum* BB 536 during refrigeration storage

The initial level of strain BB356 in all types of yoghurt were higher than 6 log CFU/ml fulfilling the requirement of probiotics foods. In control yoghurt (A) the level of strain BB 536 was 7.85 ± 0.01 Log CFU/ml. After that increased in first week of refrigeration storage to 7.93 Log CFU/ml, then decreased to 6.84 Log CFU/ml in the second weeks. These result were lower than that by Farnworth *et al.* (2006) who reported 2 Log CFU/ml increase in the first week of refrigeration storage. Similar to the control the initial growth of strain BB 536 in soymilk yoghurt (B and C) increased in the first week and then decreased in the second week.

**Table 4.5:** Survival of *Bifidobacterium longum* BB 536 (Log CFU/ml) during refrigeration storage

<table>
<thead>
<tr>
<th>Time</th>
<th>Control (A)</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yoghurt (o week)</td>
<td>7.85±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.76±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.90±0.02&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>One week</td>
<td>7.93±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.87±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.77±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Two weeks</td>
<td>6.84±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.75±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.71±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± SD; means carrying the same superscript letters in the same column are for significantly different P≤ 0.05 using minitab.

A =100% cow milk yoghurt.

B=100% soy milk yoghurt.

C=50% soymilk and 50% cow milk yoghurt.
4.4.2. pH of different yoghurt during refrigeration storage

The pH of the processed yoghurts ranged between 4.71- 5.10 as presented in Table (4.6). During the refrigeration the pH of different yoghurts decreased due to acid production. These results were lower than the value recorded by Wang et.al. (2002) which ranged from 6.13

Table 4. 6: pH of different yoghurt during refrigeration storage

<table>
<thead>
<tr>
<th>Time</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yoghurt (0 week)</td>
<td>5.10±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.71±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.80±0.12&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>One week</td>
<td>4.78±0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.38±0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.67±0.00&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Two weeks</td>
<td>4.68±0.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.22±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.34±0.00&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± SD; means carrying the same superscript letters in the same column are for significantly different P≤ 0.05 using Minitab.

A =100% cow milk yoghurt.

B=100% soymilk yoghurt.

C=50% soymilk and 50% cow milk yoghurt.
4.4.3. Moisture of different yoghurt during refrigeration storage

The moisture indifferent yoghurts increased by storage period for two weeks as presented in Table (4.7). Yoghurt C contained the lowest moisture in comparison to yoghurts A and B.

Table 4. 7: Moisture of different yoghurt during refrigeration storage

<table>
<thead>
<tr>
<th>Time</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yoghurt (0 week)</td>
<td>87.55±0.07&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>87.67±0.67&lt;sup&gt;b&lt;/sup&gt;</td>
<td>87.26±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>One week</td>
<td>86.87±0.55&lt;sup&gt;b&lt;/sup&gt;</td>
<td>87.98±0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>84.57±0.13&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Two weeks</td>
<td>88.84±0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>89.63±0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>87.63±0.49&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± SD; means carrying the same superscript letters in the same column are for significantly different P≤ 0.05 using Minitab.

A =100% cow milk yoghurt.

B=100%soy milk yoghurt.

C=50% soymilk and 50%cow milk yoghurt.
4.4.4. Total Carbohydrate of different yoghurt during refrigeration storage

Yoghurt A contained initial total carbohydrate of 5.31%, which decreased by storage period. This trend was recorded also in yoghurts B and C. The reduction of carbohydrate is due to utilization by strain BB 536. The lowest initial and final total carbohydrate was in yoghurt B (Table 4.8).

Table 4.8: Total Carbohydrate of different yoghurt during refrigeration storage

<table>
<thead>
<tr>
<th>Time</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>5.31±1.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.85±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.25±0.49&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>One week</td>
<td>3.67±0.70&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.72±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.98±0.13&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Two weeks</td>
<td>3.03±0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.46±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.59±0.52&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± SD; means carrying the same superscript letters in the same column are for significantly different P≤ 0.05 using Minitab.

A = 100% cow milk yoghurt.

B = 100% soy milk yoghurt.

C = 50% soymilk and 50% cow milk yoghurt.
4.5. Sensory characteristics of different yoghurt

The result of panelists on sensory characteristics was presented in Table . There were variation in color and texture between different types of yoghurts. However, there were no differences in color, flavor and overall acceptability between the control and soymilk yoghurts. Therefore, is possible to develop soymilk yoghurt for Sudanese people.

Table 4. 9: Sensory evaluation of yoghurt:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Color</th>
<th>Flavor</th>
<th>Taste</th>
<th>Texture</th>
<th>overall quality</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>4.25 ±0.45</td>
<td>3.66 ±1.07</td>
<td>3.41 ±1.16</td>
<td>2.50 ±1.00</td>
<td>3.66 ±0.98a</td>
</tr>
<tr>
<td>B</td>
<td>3.25 ±1.21b</td>
<td>2.83 ±1.85a</td>
<td>2.50 ±1.31a</td>
<td>3.58 ±1.24a</td>
<td>3.00 ±1.27a</td>
</tr>
<tr>
<td>C</td>
<td>3.16 ±0.71b</td>
<td>2.91 ±1.16a</td>
<td>2.66 ±1.15a</td>
<td>3.00 ±0.95ab</td>
<td>3.00 ±1.12a</td>
</tr>
</tbody>
</table>

Values are mean ± SD; means carrying the same superscript letters in the same column are for significantly different P ≤ 0.05 using Minitab.

A=100% cow milk yoghurt
B=100% soymilk yoghurt
C=50% cow milk:50% soymilk yoghurt
CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1. Conclusions

From the results obtained in this study we can conclude that the processing of soymilk yoghurt was successful. Soymilk yoghurt contained higher protein, fat, ash and carbohydrate than control yoghurt. Same time the processed yoghurt has similar sensory characteristics and was acceptable as cow milk yogurt. Further it fulfill the requirement of probiotic foods containing more than 6 log CFU/ml yoghurt the minimum number required to presence in probiotic food.

5.2. Recommendation

1- Encourage processing cow milk alternative yoghurt for vegetarian in Sudan.

2- Highlight the high nutritional value of soymilk yoghurt for consumers.

3- Optimize growth of probiotic in soymilk yoghurt.

4- Further studies required on amino acid and vitamin profiles of processed yoghurt.

5- Planting soya bean in Sudan as it has a high nutritive value.
References


Majid, S. (2016). Bioactive Components in Milk and Dairy Products, Swedish University of Agricultural, Department of Food Science, Malaysia.


Wright. D. (2016). Food Science Diary, PHD, University of Wisconsin-stout, Department of chemistry and physic, Wisconsin-stout.
APPENDICES

A = 100% cow milk yoghurt.

B=100% soy milk yoghurt.
C = 50% soymilk: 50% cow milk.

A = 100% cow milk yoghurt.
B = 100% soy milk yoghurt.
C = 50% soymilk: 50% cow milk.