

Chapter One

Introduction

Cow's milk is used for the production of fermented milks including yogurt, in a majority of the countries around the world. In the Indian subcontinent, buffalo milk and blends of buffalo and cow milk are used widely for Dahi (a type of fermented milk) making, using mixed mesophilic cultures (Aneja *et al.*, 2002). Buffalo milk is the base for making yoghurt using thermophilic cultures in several Asian countries, whereas sheep, goat and camel milk are starting materials of choice for fermented several Middle Eastern countries. Yoghurt represents a very significant dairy product worldwide in modern times, it is a semi- solid fermented product made from a heat-treated standardized milk mix by the activity of a symbiotic blend *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus* (Clark and Plotka, 2004; Ozer, 2010).

In certain countries, the nomenclature “yogurt” is restricted to the product made exclusively from the two lactic cultures, whereas in other countries it is possible to label a product as “yogurt” if it is made with yogurt cultures and adjunct probiotic cultures. The more common adjunct cultures are *Lactobacillus acidophilus*, *Bifidobacterium* SPP, *Lactobacillus gasseri* and *Lactobacillus rhamnosus* (Maity and Misra, 2009; Chandan and Nauth, 2012). Yoghurt represents the most popular fermented milk product worldwide and originates from countries around the Balkan and the Eastern Mediterranean Sea (Staff, 1998; Walstra *et al.*, 1999).

Yoghurt also has medical uses because of the probiotic characteristics, in helping out on a variety of gastro intestinal conditions and in preventing antibiotic associated diarrhea (Lourens-Hattingh and Viljoen, 2001; Mazahreh and Ershidat, 2009).

The art of making Zabadi (yoghurt) came to Sudan from Egypt, most likely during the time of the Anglo- Egyptian rule (1898-1956). It was prepared by households, in its preparation; cow's milk is boiled, cooled and inoculated by back- shopping from previous lot. It is then incubated in a warm carrier where it sours and refrigerated and consumed with sugar as a dessert or to eat with wheat bread. Sometimes it is fed to babies and often turned into sauce for porridge (Chandan, 1999).

Objectives of the study:

The objectives of the study are to determine:

1. The effect of starter cultures (*lactobacillus acidophilus* and *lactobacillus plantarum*) on the organoleptic properties of set yoghurt.
2. The effect of storage period on the chemical and sensory evaluation of set yoghurt.

Chapter Two

Literature Review

2.1. Milk:

Milk is a complete food for new born mammals during the early stages of rapid development (Shah, 2000). Milk or mammal milk is a white liquid produced by the mammary glands of mammals. It is the primary source of nutrition for young mammals before they are able to digest other types of food. Early-lactation milk contains colostrums, which carries the mother's antibodies to its young and can reduce the risk of many diseases. Milk contains many other nutrients and the carbohydrate lactose (Pehrsson *et al.*, 2000).

As an agricultural product, milk is extracted from mammals during or soon after pregnancy and is used as food for humans. Worldwide, dairy farms produced about 730 million tonnes of milk in 2011, from 260 million dairy cows. India is the world's largest producer of milk, and is the leading exporter of skimmed milk powder, yet has little to no other milk product exports (Faye and Konuspayeva, 2012).

.The ever increasing rise in domestic demand for dairy products and a large demand-supply gap could lead to India being a net importer of dairy products in the future. (Owen *et al*, 2005).

New Zealand, the European Union's 28 member states, Australia, and the United States are the world's largest exporters of milk and milk products. China and Russia are the world's largest importers of milk and milk products (Heme and Ottel, 2010).

Throughout the world, there are more than six billion consumers of milk and milk products. Over 750 million people live within dairy farming households (Pehrsson *et al.*, 2000).

2.2 Fermented milk products:

Fermented milks are sour milk products prepared from milk, whole partially or fully skimmed concentrated milk or milk substituted from partially or fully skimmed dried milk homogenized or pasteurized or sterilized and fermented by means of specific dairy starter cultures.

The origin of cultured dairy product is obscure and it is difficult to be precise about the date when they were first made. Fermented by lactic acid bacteria the growth and toxicity of anaerobic, sporeforming bacteria in the large intestine are inhibited (Gandhi, 2000).

Lactic acid is biologically active and capable of suppressing harmful microorganism .Especially putrefactive ones and so has favorable effect on human viral activities. Metchnikoff's theory of longevity considerably influenced the spread of fermented milk products to many countries, particularly in Europe he also promoted extensive studies concerning biochemical and physiological properties of fermented milks. (Gandhi,2000).

Milk fermentation for processing of milk into fermented milk products for increasing the shelf-life and having different flavor and texture characteristics have been practiced in different parts of the world. Milk has been processed into cheese, yoghurt, acidophilus milk, kefir, dahi , kumiss and various other fermented products. In the preparation of various fermented milk products, lactic starters occupy the key position as the success or failure of such products is directly related to the types of starter used. Fermented milk products are prepared by various starter cultures (Gandhi,2000).

Spoilage of fermented milk products on storage also takes place due to non – lactic contaminants. Such as spore formers, micrococcus, coliforms, yeast and molds. These undesirable organisms rapidly increase

in number when the starters are weak and the ratio of non – lactic to lactic organisms is high. Containers having a large surface of air in contact with the fermented milk accelerate the process of spoilage.

Fermented milk products are generally spoiled by yeasts and molds and also by lactic acid bacteria which may cause sour, bitter and cheesy flavor (Gandhi, 2000).

2.2.1 Cultures of fermented milk:

Various microorganisms characterize the diversity of fermented milks around the world; lactic fermentation by bacteria transforms milk into the majority of products (Salampessy, and Kailasapathy, 2011). In many countries, yogurt and fermented milks with Probiotics cultures are available. These are made with defined cultures that have been scientifically documented to display certain health benefits (Sanders, 2007; Chandan and Kilara, 2008; and Chandan , 2011). It has been estimated that about 80% of the yogurt sold in the USA contains probiotic *lactobacillus acidophilus* (Schultz, 2011).

2.2.2. Cultured butter milk:

Cultured butter milk is an important fermented milk in the USA .it is obtained from pasteurized skim or part – skim, milk cultured with lactococci and aroma- producing bacteria leuconostocs (Ahmed and Wangsai, 2007; Aneja et al., 2002).

2.2.3 Sour cultured cream:

Sour cultured cream is a significant fermented – milk product in North America. It is manufactured by culturing pasteurized cream with lactococci and aroma-producing bacteria leuconostocs. It has a butter-like aroma and flavor. Cream is standardized to 18% fat, 9% MSNF (Milk solids- not- fat). and 0.3% stabilizer to get a stable acid gel. The blend is heat treated at 72°C (162°F),. It is cooled to 22°C (72°F) inoculated with

2-5% of the starter and cultured for 16-18 hours at 22°C (72°F). until the pH drops to 4.7. It is packaged in cartons and cooled to 4°C (39-40°F) to develop a thick consistency in individual serving cups/ pack.

This product resembles sour cream, except it contains a higher proportion of fat (50%, as compared to 18% in sour cream) and has a higher PH of 6.2-6.3. Cultured cream is used as atopping on vegetables, salads, fish, meats and fruits, and as an accompaniment to Mexican meals. it is also used as adip , as afilling in cakes , and in soups and cookery items (Chandan,1997; Chandan, 1989).

2.3 Culture milk:

These are seeded but unfermented milks which deliver significant doses of Probiotics microorganisms. In this case, the growth of the culture is intentionally avoided in order to preserve the fresh taste of the milk. Accordingly, the product is maintained at refrigeration temperature at all times .In the past, acidophilus milk was marketed by fermenting sterilized milk with *lactobacillus acidophilus*. The inoculated base was incubated at 37°C (98.6°F) for 24 hours (Chandan, 2002).

The plain product developed a titratable acidity of 1-2%. Consequently, it had a very harsh, acidic flavor. Its popularity declined rapidly as sweetened yogurt with fruit flavors began to dominate the market. However, *L.acidophilus* does have a strong consumer appeal. most of the yogurts (80%) now sold in the USA contain *L.acidophilus*, which is either added after culturing with yogurt culture or is co cultured with yogurt culture . Sweet acidophilus milk is an acceptable substitute for acidophilus milk. The product is based on pasteurized and chilled low- fat milk, to which a concentrate of lactobacillus is added. (Chandan, 1999).

2.4. Scandinavian fermented milk:

Scandinavian has a high per capita consumption of fermented milks. Sweden is reported to consume 28.48kg (62.8pounds) of yogurt per person (Schultz, 2011).

There are distinctive differences in flavor and texture among fermented milks in Scandinavian countries. These are generally characterized by a ropy and viscous body. They include viili, ymer, skyr, Langfil, Kelder milk and several local products (Chandan, 2007).

2.4.1. Viili:

Villi fermented milk of Finland, is sold as a plain as well as a fruit-flavored product. Its fat content ranges from 2 to 12% milk standardized to the required fat level is heat-treated at 82-83°C (180-181°F) and held at this temperature for 20-25 minutes homogenization is avoided. It is then cooled to 20°C (39-40°F) inoculated with 4% starter, consisting of diacetyl-producing *L. Lactis subsp.lactis*, *leuc . mesenteroides subsp. cremoris* and a fungus *Geotrichum candidum*. Following packaging in individual cups, in cubation at 20 °C (29-40°F) for 24 hours results in acid development (0.9 % titratable acidity) and a cream layer on the top of the cup. The cream layer traps the fungus, giving atypical musty odor to the product (Mistry, 2001).The fermentation process also elaborates mucopolysaccharides, imparting ropiness and viscosity (Chandan, 2011).

2.4.2 Ymer:

Ymer is Danish product with characteristic high protein (5-6%) and a pleasant acidic flavor with buttery notes. Protein enrichment may be achieved by ultrafiltration technology prior to fermentation. Alternatively, the traditional process involves removal of whey by draining curd after fermentation or inducing separation of whey by heating curd and then removing it. The standardized milk base is heated to 90-95°C (194-204°F)

for 3 minutes and cooled to 20 °C (39-40°F). It is then inoculated with a mesophilic culture consisting of a blend of *LC. Lactis* subsp. *Lactis* biovar. *diacetylactis* and *Leuc. Mesenteroides* subsp. *cremoris*. After incubation at 20°C (39-40°F) for 18-24 hours, the product is cooled and packaged (Chandan and Kilara, 2008).

2.4.3 Skyr:

Skyr is another Scandinavian product. In Iceland it is obtained by fermenting yeast. A small amount of rennet may be used to develop a heavier body. The milk base is cultured at 40°C (104°F) until a pH of 4.6 is obtained (Chandan and Nauth et al., 2012).

2.5 Russian and Eastern European Fermented milk:

2.5.1 Kefir:

Kefir is relatively popular fermented milk in Russia Eastern Europe and certain Asian countries. In addition to lactic fermentation, this product employed by yeast fermentation. Thus a perceptible yeast aroma and alcohol content characterized this product. Also a fizz is noticeable due to the production of carbon dioxide, as a result of yeast growth. Kefir utilized natural fermentation of cow's milk with kefir grains. Kefir grains consist of a curd-like material, which is filtered off after each use and reused for inoculation of the next batch. Kefir grains contain polysaccharides and milk residue embedded with bacteria, lactic acid organisms and species of leuconostocs, lactococci and lactobacilli. Along with bacteria, the grains contain yeasts including *Saccharomyces kefir*, *Candida kefir* and *Torula spp.* Milk is heated to 85°C (85°F) for 3 minutes then cooled to 22°C (72°F) and incubated with kefir grains for 12-16 hours to obtain traditional kefir. Typical flavor compounds in kefir are lactic acid, acetaldehyde, diacetyl, ethanol and acetone (Chandan, et al., 1993). Kefir is now available in the USA it varies from traditional kefir in that it is fermented with a blend of

species of lactococci and lactobacilli. Some yeast is used to give only traces of alcohol. The commercial product is blended with sugar and fruit juices / flavors (Chandan and Shahani,1995).

2.5.2 Koumiss:

Koumiss is obtained from mare or cow's milk, using a more defined culture containing *lactobacillus delbrueckii subsp. bulgaricus*, *lab. acidophilus* and Torula yeasts . This therapeutic product has perceived health benefits and is recommended for all consumers, especially those with gastrointestinal problems, allergies or hypertension / ischemic heart diseases (Mistry, 2001) since mare milk has only 2% protein, no curdling is seen in the product. It contains 1.0-1.8% lactic acid, 1.0-2.5% ethanol and enough carbon dioxide to give it frothy appearance (Clark, and Plotka,2004).

2.6 Middle East Fermented Milk:

Fermented milk and their products have historically been associated with the Middle East. Fermented in earthen ware pots. The organisms responsible for fermentation are thermophilic lactobacilli in the summer and mesophilic lactococci in the winter (Mistry, 2001; Deibel, and Deibel, 2008).

2.6.1 Laban rayeb or Roub:

This is prepared at home by pouring raw whole milk into clay pots and allows the fat to rise at room temperature. The top cream is removed, and partially skimmed milk is allowed to undergo spontaneous fermentation. Some variations of the product exist. One is called laban khad, which is fermented in a goat pelt another is named laban Zeer (Mistry, 2001). Roub is a traditional fermented dairy product produced in the rural areas of the Sudan from cow's milk when plenty of milk is available during the rainy season. Roub is made from surplus unheated

milk by inoculating with starter culture from the fermentation of the previous day. After coagulation, the curd is churned early in the morning either by *Sîin* (made of tanned goat skin) or by *Bokhsa* (a gourd made from dried fruit of the plant *Lagenaria peucantha*). After the separation of the cream (locally known as *Fursa*), the remaining part is the *Roub* which is either drunk by diluting with water (called *Gobasha*) or added to powdered okra to make a soup eaten with a pudding (Abdelgadir *et al.*, 2001; Hussain, 2010).

2.6.2. Kishk:

This is obtained from laban zeer .wheat grains are soaked, boiled, sun – dried and ground into power.

The blend of wheat and laban zeer is allowed to ferment further for another 24 hours and then portioned into small lumps and sun- dried.

The dried kishk has 8%moisture and 1.85% lactic acid. After proper packaging shelf- life is of the order of several years. Kishk may contain spices (Josephsen and Jespersen., 2004).

2.6.3 Labneh:

This is prepared concentrating fermented milk after the fermentation process is completed the milk is fermented with a yogurt culture and then concentrated using a quarg separator. This product contains 7-10%fat (Goddik, 2012).

2.6.4.Zabady:

Khurana and Kanawjia, (2007) reported that Zabadi is an Egypt product obtained by fermenting milk which has been concentrated by boiling and then fermented with yogurt culture. Further concentration of milk solids is achieved by heating it and separating the whey.

2.7. South Asian fermented milks:

The following fermented milks and products derived there and are of commercial importance in India – Pakistan and Bangladesh (Aneja *et al.*, 2002; Maity and Misra, 2009).

2.7.1.Dahi:

Dahi is a semi solid product obtained from pasteurized or boiled buffalo or cow and buffalo milk by souring , natural or otherwise , by a harmless lactic acid or there bacterial culture. Dahi may contain cane sugar. It should have at minimum the same percentage of fat solids-not – fat as the milk from which it is prepared (Aneja *et al.*, 2002). Use of the right type of culture is essential to the manufacture of good – quality Dahi. A mixed culture containing *lactococcus lactis* sub sp. *lactis*, *lactococcus lactis* sub sp. *Diacetilactis* or leuconostoc species, and *lactococcus lactis* sub sp. *cremoris* in the ratio of 1:1:1 may be used. in addition *Streptococcus thermophilus* may be a component of dahi culture , or aculture composed of *lactococcus lactis* sub sp. *lactis* and *lactococcus lactis* sub sp. *diacetilactis* may be employed. Mild dahi is made from mesophilic lactococci- leuconostocs may be used as adjunct organisms for an added kuttery aroma and flavor. Sour Dahi contains additional cultures belonging to a thermophilic group (Mistry, 2001)

Which are generally thermophilic organisms grow rapidly at 37-45C (98.6-113F) producing Dahi in less than 4hours (Salampessy,,and Kailasapathy,, 2011).

2.7.2 Shrikhand:

This is a Dahi- based product resembling Greek yogurt. The cultured milk or Dahi is separated from whey to get Chakka which is blended with sugar, color, flavor and spices to reach desired level of composition and consistency. The final product contains 8.5% fat %, 10% protein, 42% sugar and 60% total solids. The acidity of the product is usually between

1.1 and 1.2% expressed as lactic acid. Skim milk has 9% MSNF, 0.05% fat (Sanders and Marco, 2010). Is heated to 90°C (194.4°F) for 10 seconds in a high- temperature short- time (HTST) pasteurizer, cooled to 30°C (86°F) and inoculated with 0.25-0.50% Dahi culture . After 8 hours of incubation or at a titratable acidity of 0.8%, the curd is ready for further processing.

Chakka is prepared by separating the whey from Dahi using a basket centrifuge, a quark separator or a desluding centrifuge. Shrikhand is prepared adding sugar at a rate of 80% of the amount of Chakka and mixed in a planetary mixer. A predetermined amount of plastic cream (80% fat) is added to the Chakka , along with sugar and flavorings / spices, to obtain at least 8.5% fat in the finished product. Shrikhand is used primarily as asnack and dessert (Tamime and Robinson, 2007).

2.8 Yoghurt:

Yoghurt and fermented milk products are generally considered as a healthy food and are among dairy products widely consumed around the world (Remeuf *et al.* 2003). Yoghurt is easily digested, has high nutritional value, and is a rich source of Carbohydrates, protein, fat, vitamins, calcium and phosphorus. Because milk protein, fat, and lactose components undergo partial hydrolysis during fermentation, yoghurt is an easily digested product of milk (Rasic and Kurmann 1978; Lee *et al.* 1988; Sanchez-Segarra *et al.* 2000). Ancient Turks who lived as nomads possibly introduced yoghurt to village people as a preserved milk product (Akin and Rice, 1994).

Yoghurt is made in a variety of compositions either plain or with added substances, fruits, sugar, gelling agents and beverages (Walstra *et al.*, 1999). The essential flora of yoghurt is thermophilic acid bacteria (*Streptococcus thermophilus* and *lactobacillus bulgaricus*). Yoghurt flora

form substances contributed in formation of flavor, consistency and structure of the product (Fox, 1993).

Fox (1993) reported that the art of making yoghurt (Zabadi) came to Sudan from Egypt, most likely during the time of the Anglo-Egyptian rule (1898-1956). It was prepared by households in its preparation; cow's milk is boiled, cooled and inoculated by back – shopping from a previous lot. It is then incubated in a warm carrier where it sours and refrigerated and consumed with sugar as a dessert or to eat wheat bread with sometimes it is fed to babies and often turned into sauce for a ceda (porridge).

2.8.1. Types of yoghurt:

There are two major types of yoghurt set and stirred.

Set yoghurt (which may include fruit on the bottom) is formed in retail pots as lactic acid bacteria ferment lactose into lactic acid giving a continuous gel structure in the consumer container. In stirred yoghurt, the acid gel formed during incubation in large fermentation tanks is disrupted by agitation (stirring), and the stirred product is usually pumped through a screen which gives the product a smooth and viscous texture (Tamime and Robinson, 1999).

2.8.2. Factors affecting the physical and sensory properties of yoghurt:

2.8.2.1. Dry matter fortification:

The physical and sensory properties of yoghurt gels are greatly influenced by the total solids content of the yoghurt milk, especially the protein content. An increase in the total solids could be obtained by the addition of skim milk powder or by ultra filtration (Biliaderis *et al.*, 1992). Increased yoghurt viscosity is observed when the total solids content of milk is increased (Guirguis *et al.*, 1984). The oral viscosity of yoghurt or

perceived thickness also increased with an increase in total solids content of milk (Sodini *et al.*, 2004). Addition of whey protein concentrates (WPC) to milk followed by high treatment led to decreased gelation time in yoghurt (Lucey *et al.*, 1999). For improvement of yoghurt texture, milk or skim milk was fortified with other materials such as non some other dairy or plant – based ingredients (Tamime and Robinson, 1985). Becker and Puhan (1989) found that the addition of 3 to 4% of NSM (Not solid milk), to yoghurt mix is common to increase total solids (TS) for low fat, non fat and low calorie yoghurt. Viscosity of yoghurt is affected by composition, type of starter cultures, heat treatment and stabilizer. As the TS increase, viscosity and firmness increase. (Tamime *et al.*, 1994).

2.8.2.2. Heat treatment:

Native whey proteins from untreated milk are inert fillers in yoghurt (Lucey *et al.*, 1999). When milk is heated at $> 70^{\circ}\text{C}$, the major whey proteins such as, B- Lactoglobulin interacts with the k-casein molecules, (i.e.k- casein that dissociates from the micelle at high temperatures) by disulfide bridging, which results in increased gel firmness and viscosity of yoghurt (Dannenberg and Kessler, 1988) . Hong and Goh (1979) found that yoghurt from milk heated at 85°C was harder than yoghurt from milk heated at 95 or 75°C and it received highest subject scores for appearance, aroma and flavor.

Culture yoghurt from UHT(Ultra-high temperature), processed whole milk (149°C for 3 sec) was lower in gel hardness and apparent viscosity but higher in spread ability and fluidity than yoghurt processed by conventional vat system (82°C for 30 min). An increase in heat treatment resulted in an increase in oral viscosity and perceived mouth coating attributes, as well as, a decrease in the chalkiness attributed to stirred yoghurt (Lee and Lucey, 2006).

2.8.2.3. Incubation temperature:

Physical properties and microstructure of yoghurt are influenced by incubation temperature. The use of high incubation temperature resulted in a decrease in gelation time at pH 4.6, an increase in whey separation compared with yoghurt gels incubated at low temperature (Lee and Lucey, 2003). Yoghurt gels formed at high temperature are weak and have a coarse gel net work due to extensive rearrangement resulting in the formation of large pores and greater whey separation (Lee and Lucey, 2004). Lee and Lucey, (2006) showed that higher viscosity was observed in stirred yoghurts that had been incubated at high temperature (e.g.>40°C). As incubation temperature increased, there was a decrease in the sensory attributes, such as mouth coating and smoothness of stirred yoghurt (Martin *et al.*, 1999).

2.8.2.4. Starter culture:

Lee *et al.* (1988) reported that the selected starters *L.bulgaricus* isolated from raw milk and *S.thermophilus* in a ratio of approximately 1:1 was much higher than commercial yoghurt starter in the acid production and growth of starter, and the yoghurt manufactured with selected starter was better than commercial yoghurt in sensory evaluation such as taste, flavor and over all acceptability . Rao *et al.* (1982) studied the preparation of yoghurt at room temperature using a mixed culture of *S.thermophilus* and *L.bulgaricus* based on coagulation time and acid production.

A ratio of 1:2 of starter *S.thermophilus* and *L. bulgaricus* gave a good quality product more lactic acid is produced by a mixed yoghurt starter culture consisting of *S.thermophilus* and *L.bulgaricus* in yoghurt prepared from lactose hydrolyzed milk (O'Leary and Woychik, 1976). Production of lactic most important chemical process which occurs during yoghurt manufacture the lactic acid helps to destabilize the casein micelle and this

leads coagulation of the milk protein and formation of yoghurt gel. The lactic acid also gives the sharp and acid taste to yoghurt and contributed to the typical aromatic flavor (Tamime and Deeth, 1980). Acetaldehyde in yoghurt was reported to be produced from lactose. An increase in total solids resulted in an increase in titratable acidity and reduction in the coagulation time (Tamime and Deeth, 1980).

The largest amount of acid was produced by *S.thermophilus* in milk heated at 65 °C for 30 min. While *L.bulgaricus* produced the maximum amount of acid in milks heated at 85°C for 10 minutes in the mixed culture, increased acid and acetaldehyde production was noted in milk samples steamed for 30 min (Singh *et al.*, 1980).

2.9. Health benefits of yoghurt:

Some strains of *L. acidophilus* have been studied extensively for health effects. The Mayo clinic publishes a list of disorders for which *L.acidophilus* has been tested, grading the evidence for each use from strong evidence of effectiveness, through unclear, down to strong evidence of ineffectiveness.

According to the list there is good (rather than strong) evidence supporting the use of *L.acidophilus* or yogurt enriched with it for the treatment of some vaginal infections, effectiveness for other conditions ranges from unclear to fair negative evidence some *L. acidophilus* strains may be able to survive gastrointestinal transit, being resistant to bile, low pH, and digestive enzymes. They may then be able to adhere to human epithelial cell lines and human intestinal mucus.

L.acidophilus led to a significant decrease in levels of toxic amines in the blood of dialysis patients with small bowel bacterial over growth. At adequate daily feeding levels, *L.acidophilus* may facilitate lactose digestion in lactose – intolerant subjects (Singh *et al.*, 1980).

A University of Nebraska study found that feed supplemented with *L.acidophilus* ,fed to cattle resulted in a 61% reduction of *Escherichia coli* 0157:H7. The research indicated that *L.acidophilus* may be helpful reducing serum cholesterol levels (Singh *et al.*, 1980).

2.10. Storage of yoghurt:

Singh *et al.* (1980) reported that initial taste of the stirred yoghurt was maintained during storage for 16 days at 5°C and the higher the storage temperature, the faster were the rate of titratable acidity, PH and viable cells of lactic acid bacteria. The shelf- life of any food commodity should combine the two consideration of safety and organoleptic property of the product. It is the latter requires emphasis by the dairy industry before attempts are made to prolong the shelf – life of plain liquid yoghurt beyond five days (Salji *et al*, 1984).

2.11. Yoghurt microbiology:

Yoghurt bacteria are now characterized as lactic acid bacteria belong to lactobacillaceae and streptococcaceae genera. Generally, yoghurt cultures are *L. delbruecki subspbulgaricus* and *S.thermophilus* which are thermoduric, homofermentative lactic acid bacteria (Tamimme and Deeth, 1980). Some other strains such as *L.helveticus*, *L.jugurti*. *L.acidophilus* and *bifidobacterium spp.* are used as adjuncts when a single strain of either *L.delbrueckii sub spbulgaricus* or *S.thermophilus* is used. Lactic acid and acetaldehyde production is lower compared with that in a mixed culture (Hamdan *et al.* 1971).

There are two stages involved in yoghurt fermentation. In the first stage *L.delbrueckii sub sp. bulgaricus* stimulates the growth of *S. thermophilus* by liberating essential amino acids from casein by proteolytic activity (Sandine and Elliker, 1970) .In this stage L. delbrueckii subsp. bulgaricus grows slowly because it is microaerophilic.At the end of the

first stage, the growth of *S.thermophilus* is slowed down because of the high lactic acid concentration. When *S.thermophilus* produces enough formic acid, which stimulates growth of *L. delbrueckii sub L. bulgaricus*, the second stage begins by *L.delbrueckii subsp. bulgaricus*, by this symbiotic action, the desirable acidity of the final yoghurt could be achieved (Rasic and Kurmann, 1978).

Lactobacillus delbrueckii sub sp. bulgaricus and *S.thermophilus* are proteolytic bacteria. The former has a higher proteolytic activity. Slocum *et al.*(1988) reported that in yoghurt with 10.0 to 17.5% total solid (TS) maximum proteolysis occurred at 14.5% TS . Although. There are no regulatory requirements in U.S regarding the number of viable *L.delbrueckii sub sp. bulgaricus* and *S.thermophilus*. It has been established with respect to therapeutic properties that yoghurt should contain live lactic acid bacteria (Roberts and Maust, 1995). In countries such as Japan, South Korea and Poland , legislation requires viable lactic acid bacteria in the final product ranging from 10^6 to 10^8 cells/g (Hamann and Marth 1984; Orihara et al.,1992). Strains of *L.delbrueckii subsp. bulgaricus* and *S.thermophilus* have been studied and used to produce smooth and viscous yoghurt (Hess *et al.*, 1997).

2.12. *Lactobacillus acidophilus*:

L. acidophilus is a homofermentative, microaerophilic species, fermenting sugars into lactic acid, and grows tardily at rather low PH values (below pH 5.0) and has an optimum growth temperature of atound 37°C (99°F)*L.acidophilus* occurs naturally in the human and animal gastrointestinal tract and mouth . some strains of *L.acidophilus* may be considered to have probiotic characteristics .These stains are commercially used in many dairy products, sometimes together with *Streptococcus thermophilus* and *lactobacillus delbrueckii subsp. bulgaricus* in the production of acidophilus- type yogurt. (Holmes *et al.*,2001).

Apart of the claims in favour of such treatment refer to attaining a better digestion. *L.acidophilus* LA-5 produces bacteriocin (CH5) that is both antibacterial and inhibitory against certain yeasts molds and is effective against both *Salmonella typhimurium* and *Campylobacter jejuni*. It has been shown to improve bowel regularity and has been shown to have a preventative effect against traveler's diarrhea, as well as antibiotic-related bowel issues (Holmes *et al.*,2001).Because of its relation to gut – associated lymphoid tissue (GALT), *L.acidophilus* (LA-5) has been associated with positive effects on the immune system such as increased cytokine , phagocytic activity and antibody production , as well as phagocytosis of salmonella , and *L. acidophilus* NCFM has even been shown to reduce incidence of symptoms of fever , cough and runny nose . (Holmes *et al.*, 2001).

2.13. *Lactobacillus Plantarum*:

Lactobacillus plantarum is widespread member the genus lactobacillus, commonly found in many fermented food products as well as anaerobic plant letter. It is also present in saliva (from which it is first isolated). It has the ability to liquefy gelatin. *L. plantarum* has one of the largest genomes known among the lactic acid bacteria and is a very flexible and versatile species. (Juana *et al.* (2008).

2.13.1. Metabolism:

L. Plantarun is a Gram-positive- aero tolerant bacteria that grows at 15 c° (59 F°) but not at 45°C (113 F°) and produces both isomers of lactic acid (D and L). (Juana *et al.* (2008) .studied; *L. plantarum* has been applied to reduce the allergenic of soy flour. The result showed that, compared to other microbes, *L plantarum* fermented soy flour showed the highest reduction ige immunoreactivty (96 – 99%), depending upon the sensitivity of the plasma used. *L. plantarum* is also found in dadiah, a traditional fermented buffalo milk of Minangkabau tribe Indonesia.

2.13.2. Therapeutics:

L. plantarum has significant antioxidant activities and also helps to maintain the intestinal permeability. It is able to suppress the growth of gas producing bacterium in the intestines and may have benefit in some patients *Lactobacillus plantarum* has been found in experiments to increase hippocampal brain derived neurotrophic factor (Kleerebezem *et al.*, 2003).

2.14. Packaging Materials:

Packaging materials are essential to protect the food product and to preserve its inherent quality during the storage.

The interactions between food constituents and packaging materials may alter the sensory quality of the product:

1. The interactions occurring during storage are mainly Sorption phenomena from food to packaging materials
2. Migration phenomena from packaging to the food.

The sorption of flavor compounds is influenced by the properties of the polymers and the flavor molecules.

And by the external conditions of storage can cause a fall in the acidity of fresh dairy products and an increase in yoghurt viscosity (Van Willige *et al.*, 2001).

2.15. Probiotics:

Probiotics are live bacteria that may confer a health benefit on the host. More than 20 years ago, Fuller (1989) defined Probiotics as live microbial feed supplements which beneficially affect the host animal by improving its intestinal microbial balance. The term Probiotics is currently used to name ingested microorganisms associated with beneficial effects to humans and animals (Magdalena *et al.*, 2006). 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 (Gismondo *et al.*, 1999). The dependence of the intestinal microbes on the food makes it possible to adopt measures to modify the flora in our bodies and to replace the harmful microbes by useful microbes (Slashinski *et al.*, 2012).

A significant expansion of the potential market for probiotics has led to higher requirements for scientific substantiation of putative beneficial effects conferred by the microorganisms (Rijkers *et al.*, 2011). Probiotics have received renewed attention recently from product manufacturers, research studies and consumers. The history of Probiotics can be traced back to the first use of cheese and fermented products, that were well known to the Greeks and Romans who recommended their consumption (Gismonodo *et al.* .1999).

Chapter Three

Material and Methods

This study was conducted during the period October – November 2014 at the Department of Dairy Sciences and Technology, College of Animal Production Science and Technology, Sudan University of Science and Technology.

3.1. Materials:

Eight liters of cow's raw milk as samples were purchased from the Dairy Farm, College of Animal Production Science and Technology, Sudan University of Science and Technology, Hillat Kuku, sterilized containers were used for the collection of samples. Starter cultures of yoghurt (*S. thermophilus* and *L. bulgaricus*) and adjunct starter culture of *L. acidophilus* and *L. plantarum* (1:1) were brought from Vitane pharma GmbH 82515 Wolfratshausen Germany.

3.2. Adjunct starter culture preparation:

One liter of skim milk was sterilized at 85 °C for 30 min and cooled to 40 – 45 °C then inoculated with the adjunct starter culture at the rate of 2% and incubated at 45°C until coagulation occurred.

3.3 .The starter culture:

Conventional yoghurt , and adjunct starter cultures were added at the rate of 2% of the milk used for yoghurt making.

3.4. Methods:

3.4.1. Yoghurt making process:

Yoghurt was prepared as described by Staff (1998), eight liters of cow's raw milk samples were heated in a water bath at 85 °C for one hour and cooled to 45°C , then were divided into four portions, the first

portion was used as control only with yoghurt starter culture. To the 2nd , 3rd and 4th portions 50%, 75% and 100% of adjunct starter culture (*L.acidophilus and L.plantarum*) was added to each respectively and packed into plastic cups (200 mg capacity) in triplicates for each treatment and then incubated at 43°C for 3 hrs. There after samples from different treatments were stored at refrigerator 4 °C for 1, 5 and 10 days. Chemical and sensory evaluation of the yoghurt samples were analyzed for the determined period.

3.4.2. Chemical Analysis:

3.4.2.1. Fat content:

The fat content was determined by Gerber methods according to (Bradly *et al* . 1992) as follows:

In a clean dry Gerber tube, 10ml of sulphuric acid (density 1.8 gm / ml at 20 °C) were poured, and then 10.94 ml of milk sample were added, amyle alcohol (1-2ml) was added to tube, followed by the amount of distilled water. The contents were thoroughly mixed till no white particles could be seen. The Gerber tubes were centrifuged at 1100 revolution per minute (rpm) for 4-5 min . The fat column was then read immediately.

3.4.2.2. Protein contents:

The protein content was determined by kjeldahl methods according to AOAC (1990) as follows:

Digestion: ten ml of milk were weighed and poured in added. Concentrated sulphuric acid (25ml) was added to the flask. The flask were heated until a clear solution was obtained flasks were removed and allowed to cool.

Distillation: the digested sample was poured in volumetric flask (100 ml) and diluted to 100ml with distilled water. Five milliliters were distilled using 10 ml of 40% NaOH. The distillate was received in a conical flask

(100ml) containing 25 ml of 2% boric acid plus 3drops of indicator (bromcresol green + phenolphthalein red). The distillation was continued until the value in the flask was 75 ml, then the flask was removed from the distillator. Titration: the distillate was titrated with 0.1N HCL until the end point (red colour) was obtained . The protein content was calculated from the following equation.

$$\text{Nitrogen \%} = \frac{T \times 0.1 \times 20 \times 0.014 \times 100}{w}$$

$$\text{Protein \%} = \text{N\%} \times 6.38$$

Where: T= Titration figure

W = Weight of the original sample

0.1 N = Normality of HCL

0.014 = the atomic weight of nitrogen / 100

20 = Dilution factor

3.4.2.3. Total solids (T.S) content:

The total solids content was determined according to the modified methods of AOAC (1990) .Three grams of sample were weighed into a dry oven flat bottomed aluminum dish, and heated on steam bath for 10-15 min. The dish was placed in an oven at 105 C over night, and then cooled in desiccators and weighted quickly.. Weighting were repeated until the difference between the two readings was < 0.1mg. The total solids content was calculated from the following equation.

$$\text{T.S\%} = \text{W1/Wo} \times 100$$

Where: W1 = Weight of sample after drying

Wo = Weight of sample before drying

3.4.2.4. Ash Content:

The ash content was determined according to AOAC (1990). Five grams of the sample were weighed into a suitable crucible and evaporated to dryness on steam bath. Then placed in a muffle furnace at 550-600C

until ashes were carbon free (2-3 hrs), then crucibles were cooled in a desiccator and weighed the ash content was calculated from the following equation:

$$\text{Ash\%} = \frac{W_1}{W} \times 100$$

Where: w_1 = Weight of ash

W = weight of sample

3.4.3. Sensory evaluation:

Sensory profiling of the yoghurt sample was conducted, using conventional profiling, by untrained panelists according to Larmond (1977). Ten panelists were selected among staff and students of the College of Animal production Science and Technology, Sudan University of Science and Technology. The panelists were given a hedonic questionnaire (Appendix NO1) to evaluate taste texture, colour, and flavor and overall acceptability of coded samples of cow milk yoghurt. Those stored for different periods of time (1, 5, 10 days). They were scored on a scale of 1-7 (1=not acceptable, 7= acceptable). Each attribute was evaluated in triplicate and the values were then averaged

3.4.4. Statistical analysis:

Statistical analysis were carried out with SPSS (2008) version 17. General linear model was used for data analysis (Factorial design) to test the effect of adjunct starter culture addition and storage period on the quality of set yoghurt. Least significant difference (LSD) was used for mean separation between the treatments. Alpha level 5% was used in this study.

Chapter Four

Results

4.1. Effect of different levels of adjunct starter culture on the chemical characteristics of set yoghurt

Data in Table (1) showed the effect of starter culture on the physicochemical composition of set yoghurt. The results indicated that addition of adjunct starter culture had significant ($P < 0.01$) effect on the fat content of set yoghurt (Table1). The highest fat content ($3.56 \pm 0.06\%$) was for the control yoghurt, while the lowest value ($3.28 \pm 0.06\%$) was for the one with 75% adjunct starter culture.

The titratable acidity of the yoghurt samples showed significant ($P < 0.05$) variations due to the different levels of adjunct starter culture addition (Table1). As the level of adjunct starter culture increase the titratable acidity decrease. However the lowest acidity ($0.94 \pm 0.02\%$) was for the yoghurt with 75% adjunct starter culture.

Statistical analysis revealed that adjunct starter culture had no significant ($P > 0.05$) effect on the Total solids and ash contents of the set yoghurt (Table1).

The results of the study demonstrated that (Table1) addition of the adjunct starter culture had significant effect on the crude protein content. The highest protein content ($5.52 \pm 0.05\%$) was for the yoghurt with 50% each conventional and adjunct culture, whereas the lower one was for the yoghurt with 75% adjunct starter culture.

Table (1) Effect of different levels of starter cultures on chemical characteristics of set yoghurt

Treatments	Chemical Composition %				
	Fat	Acidity	T.S	Ash	Protein
A	3.56± 0. 06 ^a	1.20±0.02 ^b	12. 38±0.05	0. 75±0.02	5. 33± 0.5 ^b
B	3.51±0.06 ^b	1.23±0.02 ^a	12. 40± 0.05	0.80±0.02	5.52±0.05 ^a
C	3.28±0.06 ^d	0.94±0.02 ^d	12.35± 0.05	0. 80±0.02	4.48±0.05 ^d
D	3.43±0.06 ^c	0.99±0.02 ^c	12.51± 0.05	0. 80±0.02	5. 03±0.06 ^c
Sig	*	**	NS	NS	**

Means with different superscript in the same column are significantly(p<0.05) different

A = control yoghurt with conventional culture

B = yoghurt with 50% conventional starter and 50% adjunct cultures

C = yoghurt with 75% adjunct cultures and 25% conventional starter

D = yoghurt with 100% adjunct cultures

4.2. Effect of storage period on the chemical composition of set yoghurt:-

Data in Table (2) shows the effect of storage period on the chemical composition of the set yoghurt.

The results indicated that the storage period had significant ($p < 0.05$) effect on the fat content of set yoghurt (Table 2). The highest fat content ($3.62 \pm 0.05\%$) was at day 1. The lowest value ($3.28 \pm 0.05\%$) was at day 5.

The titratable acidity of the yoghurt samples showed significant ($p < 0.05$) variations due to the storage period (Table 2). As the storage period progressed the titratable acidity increased. However, the highest acidity ($1.21 \pm 0.02\%$) was at day 10.

The results of the study demonstrated that (Table 2) the storage period had significant effect on total solids the highest total solids ($12.52 \pm 0.04\%$) was at day 5. The lowest value ($12.30 \pm 0.04\%$) was at day 10.

Statistical analysis revealed that storage period had no significant ($p > 0.05$) effect on ash content of the set yoghurt (Table 2).

The results indicated that the storage period had significant ($p < 0.05$) effect on the crude protein content. The highest protein content ($5.97 \pm 0.05\%$) was at day 1. The lowest value ($4.96 \pm 0.05\%$) was at day 5.

Table (2) Effect of the storage period on the chemical characteristics of set yoghurt

Storage/days	Chemical Composition %				
	Fat	Acidity	T.S	Ash	C.P
Day 1	3.62± 0. 05 ^a	0.97± 0.02 ^b	12. 40± 0.04 ^b	0. 79± 0. 02	5. 97± 0.05 ^a
Day 5	3.28±0.05 ^c	1.09±0.02 ^c	12. 52± 0.04 ^a	0. 79±0.02	4.84±0.05 ^c
Day 10	3.43±0.05 ^b	1.21±0.02 ^a	12.30± 0.04 ^b	0. 79±0.02	4.96±0.05 ^b
Sig	**	**	**	NS	**

Means with different superscript in the same column are significantly (p<0.05) different

4.3. Effect of storage period and levels of adjunct starter cultures on chemical composition of yoghurt:

The results indicated that the storage period and different levels of adjunct starter cultures had significant ($p < 0.05$) effect on the fat content of set yoghurt (Table 3). The highest fat content ($3.9 \pm 0.15\%$) was for the control yoghurt on day 1. The lowest value ($2.8 \pm 0.15\%$) was for the one with 75% adjunct starter culture at day 5.

The results of the study showed that (Table 4) addition of the adjunct starter culture and the storage period had significant ($p < 0.05$) effect on the titratable acidity of the yoghurt samples, the highest titratable acidity ($1.4 \pm 0.03\%$) was for the one with 50% adjunct starter culture on day 10. The lowest value ($0.56 \pm 0.05\%$) was for the one with 75% adjunct starter culture at day 1.

The results revealed that the storage period and different levels of adjunct starter cultures had significant ($p < 0.05$) effect on the total solids of yoghurt samples (Table 5) the highest total solids content ($12.9 \pm 0.05\%$) was for the one with 100% adjunct starter culture at day 5, the lowest value ($12.2 \pm 0.20\%$) was for the one with 50% adjunct starter culture at day 10.

The storage period and different levels of adjunct starter cultures had significant ($p < 0.05$) effect on the ash of yoghurt (Table 6). The highest ash content ($0.81 \pm 0.02\%$) was for the one with 75% adjunct starter culture on 5 day, the lowest value ($0.73 \pm 0.15\%$) was for the control at day 5.

Table (3) Effect of the storage period and different levels of adjunct starter cultures on the fat content (%) of yoghurt

Treatment	Storage Period			Sig
	day1	Day5	Day 10	
A	3.9± 0.15	3.5± 0.10	3.3± 0.00	**
B	3.7±0.17	3.3±0.06	3.6±0.30	
C	3.3±0.26	2.8±0.15	3.7±0.30	
D	3.6±0.00	3.5±0.11	3.1±0.7	
Sig	**			

A = control yoghurt with conventional culture

B = yoghurt with 50% conventional starter and 50% adjunct cultures

C = yoghurt with 75% adjunct cultures and 25% conventional starter

D = yoghurt with 100% adjunct cultures

Table (4) Effect of storage period and different levels of adjunct starter cultures on the acidity (%) of yoghurt

Treatment	Storage Period			Sig
	Day 1	Day 5	day 10	
A	1.1± 0.05	1.2± 0.05	1.3± 0.01	**
B	1.1±0.00	1.1±0.01	1.4±0.03	
C	0.56±0.05	1.1±0.02	1.1±0.18	
D	1.1±0.04	0.86±0.01	1.0±0.01	
Sig	**			

A = control yoghurt with conventional culture

B = yoghurt with 50% conventional starter and 50% adjunct cultures

C = yoghurt with 75% adjunct cultures and 25% conventional starter

D = yoghurt with 100% adjunct cultures

Table (5) Effect of storage period and different levels of adjunct starter cultures on Total solids of yoghurt.

Treatments	Storage Period			
	day 1	day 5	day 10	Sig
A	12.4± 0.05	12.4± 0.05	12.3± 0.21	**
B	12.5±0.25	12.6±0.15	12.2±0.20	
C	12.5±0.08	12.3±0.05	12.4±0.05	
D	12.4±0.15	12.9±0.05	12.3±0.03	
Sig	**			

A = control yoghurt with conventional culture

B = yoghurt with 50% conventional starter and 50% adjunct cultures

C = yoghurt with 75% adjunct cultures and 25% conventional starter

D = yoghurt with 100% adjunct cultures

Table (6) Effect of storage period and different levels of adjunct starter cultures on ash content (%) of set yoghurt

Treatment	Storage period			
	Day 1	Day 5	Day 10	Sig
A	0.75± 0.05	0.73± 0.15	0.77± 0.05	**
B	0.80±0.00	0.80±0.00	0.80±0.00	
C	0.80±0.00	0.81±0.02	0.80±0.00	
D	0.80±0.00	0.80±0.02	0.80±0.00	
Sig	**			

A = control yoghurt with conventional culture

B= yoghurt with 50% conventional starter and 50% adjunct cultures

C=yoghurt with 75% adjunct cultures and 25% conventional starter

D=yoghurt with 100% adjunct cultures

Table (7) Effect of the storage period and different levels of adjunct starter cultures on protein contents of set yoghurt

Treatments	Storage period			
	Day 1	Day 5	Day 10	Sig
A	5.6± 0.07	4.9± 0.49	5.5± 0.04	**
B	5.1±0.07	5.4±0.07	6. 0±0.08	
C	5.2±0.07	4.0±0.04	4.2±0.07	
D	5.9±0.07	5. 0±0.07	4.1±0.05	
Sig	**			

A = control yoghurt with conventional culture

B = yoghurt with 50% conventional starter and 50% adjunct cultures

C = yoghurt with 75% adjunct cultures and 25% conventional starter

D = yoghurt with 100% adjunct cultures

The storage period and different levels of adjunct starter cultures had significant ($p < 0.05$) effect on the crude protein content of set yoghurt (Table 7) the highest protein content ($6.0 \pm 0.08\%$) was for the one with 50% adjunct starter culture on 10 day, the lowest value ($4.0 \pm 0.04\%$) was for the one with 75% adjunct starter culture at day 5.

4.4. Effect of adjunct starter culture on the sensory characteristics of set yoghurt:-

Addition of adjunct starter culture had significant ($p < 0.01$) effect on the color of set yoghurt (Table 8), the highest value (8.07 ± 0.34) was for the control yoghurt while the lowest one (6.80 ± 0.34) was for the one with 100% adjunct starter culture.

The results showed that adjunct starter culture had significant ($p < 0.05$) effect on the flavor of set yoghurt (Table 8). The highest scores (7.20 ± 0.28) was for the one with 75% adjunct starter culture, however, the lowest value (5.60 ± 0.28) was for the one with 100% adjunct starter culture.

The Texture and taste of the yoghurt samples were found to be affected significantly ($p < 0.05$) by the adjunct starter culture addition. The highest texture value (7.33 ± 0.32) was for the one with 50% adjunct starter culture and the lowest value ($5.07 \pm 0.22\%$) was for the one with 100% adjunct starter culture. The highest taste scores (6.87 ± 0.41) was for the control yoghurt and the one with 50% adjunct starter culture, the lowest value (4.87 ± 0.41) was for the yoghurt with 100% adjunct starter culture.

Overall acceptability of set yoghurt samples (Table 8) significantly ($p < 0.05$) affected by the adjunct starter culture addition, the highest score (7.53 ± 0.25) was for the one with control yoghurt. The lowest value (5.93 ± 0.25) was for the one with 100% adjunct starter culture.

Table (8) Effect of the different levels of adjunct starter cultures on sensory evaluation of set yoghurt

Treatment	Sensory Attributes				
	Color	Flavor	Texture	Taste	Overall
A	8.07± 0. 34 ^a	7.00± 0.28 ^a	7. 6± 0.32 ^a	6. 87± 0.41 ^a	7. 53± 0.25 ^a
B	8.00±0.34 ^a	7.07±0.28 ^a	7. 33±0.32 ^a	6. 87±0.41 ^a	7. 47±0.25 ^a
C	7.53±0.34 ^a	7.20±0.28 ^a	6.27±0.32 ^b	6.40±0.41 ^a	7.4±0.25 ^a
D	6.80±0.34 ^b	5. 60±0.28 ^b	5.07±0.22	4.87±0.41 ^b	5.93±0.25 ^b
Sig	*	**	**	**	**

Means with different superscript in the same Column are significantly(p<0.05) different

A = control yoghurt with conventional culture

B= yoghurt with 50% conventional starter and 50% adjunct cultures

C=yoghurt with 75% adjunct cultures and 25% conventional starter

D=yoghurt with 100% adjunct cultures

4.5. Effect of storage period on the sensory characteristics of set yoghurt:-

The data in Table (9) showed that no significant $P<0.05$) variations were observed in the colour, flavor and taste of the yoghurt samples. However significant differences ($P<0.05$) were in the texture and overall acceptability of the yoghurt samples.

The color of the yoghurt samples did not changed during the storage period, the highest color scores (7.95 ± 1.43) were at day 5 and the lowest value was at day 1.

The flavor of the yoghurt samples improve slightly during the storage period as the storage period progressed the flavor scores increased the highest scores (7.05 ± 1.39) were at day 10.

Storage period affected the texture of the yoghurt samples significantly ($P<0.05$). The texture scores of the yoghurt samples increased with the advancement in storage, therefore the highest texture scores (7.40 ± 1.93) were recorded at day 10.

Slight improvement in the taste of the yoghurt samples was noticed as the storage period progressed. At day 10 the taste scores (6.70 ± 2.42) was maximum.

The results indicated that the storage period affected the overall acceptability significantly ($P<0.05$). The lowest overall acceptability scores (6.75 ± 1.58) were at day 5 while the highest ones (7.60 ± 1.58) were at day 10.

Table (9) Effect of storage period on the sensory characteristics of set yoghurt

Storage period/day	Sensory attributes				
	Color	Flavor	Texture	Taste	Overall
Day 1	7.95±1.43	6.35±1.45	6.20±1.91 ^b	6.30±2.19	6.90±1.42 ^b
Day 5	7.30±2.10	6.75±1.98	6.10±2.02 ^{cb}	5.75 ±2.42	6.75±1.58 ^{cb}
Day 10	7.55±2.02	7.05 ±1.39	7.40 ±1.93 ^a	6.70±2.42	7.60 ±1.58 ^a
Sig	NS	NS	*	NS	*

Means with different superscript in the same column are significantly ($p < 0.05$) different

Chapter Five

Discussion

The addition of adjunct starter culture to the yoghurt improved its quality. The results showed (Table1) that the chemical composition of the yoghurt samples significantly ($P < 0.05$) affected by the addition of adjunct starter cultures (*L.acidophilus* and *L.plantarum*). The decrease in the fat content of the yoghurt samples with adjunct starter culture could be due to their lipolytic activities. These findings are in accordance with the results of Mutlu and Guler (2005) who observed that the fat content of bioyoghurt ranged from 3.1 to 4.5% during storage.

The titratable acidity of the yoghurt samples with adjunct starter culture showed higher values this might be due to the high potentiality of adjunct starter bacteria to convert lactose to lactic acid which increase the acidity of yoghurt samples or probably due to its lower buffering capacity and higher content of non protein nitrogen. These results are in agreement with those reported by Abrahamsen, *et al.* (1991); Salvador and Fiszman (2004).

The protein content of the yoghurt samples with 50% adjunct starter culture is higher in comparison to the other treatments (Table1). The high protein content could be due to preservative effect of adjunct culture on the protein content. Our findings are in line with the results of Hassan and Amjad (2010) and Janhoj *et al.* (2006) who showed that the protein contents of low fat stirred yoghurt ranged from 3.4-5.6%.

The Total solids and the ash contents of the yoghurt samples were not affected by the adjunct starter culture addition (Table 1). This result is not in accordance with those of Hassan and Amjad (2010) who reported

the total solids of set yoghurt with different levels of starters increased up to $15.60\% \pm 0.56\%$. The insignificant increase in ash contents was because of the loss of CO₂ and water during mix of yoghurt samples. Moreover the ash content of yoghurt samples in this study is lower than those of Akin and Guler (2005) who reported the ash value of probiotic yoghurt as 0.95%, this could be due to the action of *L.pantarum*.

The storage period affected the chemical composition of the set yoghurt significantly ($P > 0.05$) except the ash content (Table 2).

The Fat content was highest at day 1 then decrease at day 5 this could be due to the lipolytic activities of the starter cultures. These results were in accordance with those of Salji *et al.* (1984).

The results (Table 2) showed that acidity tends to increase within the 10 day-storage period. The acidity of the yoghurt samples increased significantly as the storage period progressed this could be due to the breakdown of lactose into lactic acid by the starter cultures and may be due to the lower buffering capacity of *Lactobacillus acidophilus* and higher content of non protein nitrogen and vitamins which are needed for fast growing microorganisms. These findings are inline of those reported by of Nighswonger *et al.*, 1996; Salvador and Fiszman, 2004).

The total solids of the yoghurt samples increased till day 5 (Table 2) then decreased at day 10. This probably due to the loss of moisture and the proteolytic activities of the starter cultures during storage. These results were in accordance with the findings of Hassan and Amjad (2010) who reported that total solids increased up to $15.60\% \pm 0.56$ and with those of Abubakar *et al.* (2005).

The ash contents in this study did not affect significantly by storage period (Table 2) these results are not in agreement with those of Akin and Guler (2005) who reported the ash value of 0.95%.

The storage period affected the protein content (Table 2) of the yoghurt samples significantly ($P < 0.05$) the decrease in protein % could be due to the proteolytic action of the cultures. Similar findings were reported by Janhoj *et al* (2006) who showed that the protein contents of low fat stirred yoghurt ranged from 3.4-5.6%.

The interaction between the storage period and the addition of adjunct starter cultures significantly affected the chemical composition of the yoghurt samples in all treatments (Tables 3, 4, 5, 6 and 7). The lowest fat % was for the yoghurt sample with 75 % adjunct starter culture at day 5. However the highest fat % was for the control yoghurt samples. This could be due to lipolytic nature of the adjunct starter cultures that tends to decrease the fat % in the yoghurt samples.

The mean sensory scores of the organoleptic evaluation and acceptability for the different yoghurt samples are shown in Table 8. The statistical analysis revealed that there were significant differences ($p < 0.05$) among the yoghurt samples in the sensory attributes observed due to the use of adjunct cultures.

Adjunct starter cultures affected the colour of the yoghurt samples negatively as the level of the adjunct starter cultures increase the colour scores deteriorate this could be due to the high lipolytic and proteolytic activities of the adjunct cultures. These results are in agreement with those of Nasur (2001). The flavour, texture and taste followed the same trend. The best flavour and texture scores were for the control and yoghurt with 50% adjunct starter cultures, the improvement in flavour was probably due to the effect of high lactic acid contents which control the growth of desirable organisms that produce flavour compounds as the result of sugar and protein and fat degradation.

The storage period was not found to affect the sensory characteristics of the yoghurt samples (Table 9) significantly ($P>0.05$) except the texture and over all acceptability. Our result was not in line with those of Nuser (2001). The improved in texture till the day 5 likely was due to the effect of proteolytic agents on the protein breakdown resulted in changing the structure of protein matrix and the texture become soft, compact, while the deterioration in texture thereafter at days 10 might be attributed to further hydrolysis of protein at later stages which leads to very fine and mealy structure. The colour of the yoghurt in this study was not affected by the storage period.

Chapter Six

Conclusion and recommendation

6.1. Conclusion:

Based on the results of the study the following conclusions were drawn:

- The quality of yoghurt was relatively improved with the addition of *L.acidophilus* and *L.plantarum*.
- Addition of different levels of adjunct starter culture improved sensory evaluation (overall acceptability).
- Significant variations in the sensory characteristics and chemical composition of the yoghurt with Probiotics were found.
- Storage period has no significant effect on the sensory characteristics of yoghurt.

6.2 Recommendations:

- * Further work will be required for the production of bioyoghurt from different type of Probiotics.
- * Production of Probiotics yoghurt as a new kind of dairy products must be adopted.
- * More studies about evaluation of microbiological quality of Probiotics yoghurt.

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APPENDIX

Sensory evaluation:

Sample No.	Colour	Flavour	Texture	Taste	Overall
1					
2					
3					
4					

Keys

Colour:

Acceptable	7
Slightly accept	5
Moderately accept	3
Not acceptable	1

Flavour:

Extremely intense	9
Intense	7
Moderately intense	5
Slightly intense	3
Poor	1

Texture:

Very soft	9
Soft	7
Slightly soft	5
Tough	3
Very tough	1

Overall:

Extremely intense	9
Intense	7
Moderately intense	5
Slightly intense	3
Poor	1

