بسم الله الرحمن الرحيم

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College of Graduate Studies

Effect of probiotics (*Lactobacillus acidophilus and Lactobacillus plantarum)* on physico-chemical and sensory characteristics of Sudanese white cheese

أثر إضافه المعاونات الحيويه (اللاكتوباسلس أسيدوفلس و لاكتوباسلس بلانتارم) علي الخصائص الفيزيوكيمائيه و التقييم الحسي للجبنه البيضاء السودانيه

By

ELGILANI ABDALHADI MOHMMED AHMED ABUALKHYRAT

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Supervisor:

Prof. Dr. Omer Ibrahim Ahmed Hamid

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بِسْمِ ٱللَّهِ ٱلرَّحْمَٰنِ ٱلرَّحِيمِ

قَالَ تَعَالَى:

﴿ وَإِنَّ لَكُمْ فِي ٱلْأَنْعَٰمِ لَعِبْرَةً نُّسْقِيكُم مِّمَّا فِي بُطُونِهِۦ مِنۢ بَيْنِ فَرْثٍ وَدَمٍ لَّبَنًا خَالِصًا سَآئِغًا لِّلشَّٰرِبِينَ ﴿٦٦﴾ ﴾

صدق الله العظيم

الآية ٦٦ من سورة النحل

DEDICATION

I dedicate this work to:

my Mother

my father

my brothers and sister

my colleagues

to you . . .

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Praise thanks to Allah who gave me the health, strength and patience to conduct this study. I indebted to my supervisor prof. Dr. Omer Ibrahim Ahmed Hamid, for his guidance, close supervision, valuable suggestions, continuous advices and patience. These are few words to thanks him for what he has done during this study.

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Abstract

This study was carried out in the milk processing units at College of Animal Production Science and Technology, Sudan University of Sciences and Technology during May-June (2017). The chemical and sensory characteristics of Sudanese white cheese were investigated as affected by different levels of probiotics (*Lactobacillus acidophilus and Lactobacillus plantarum*) and storage period. Thirty liters (30) of cow's milk purchased from dairy farm, of College of Animal Production Science and Technology. The milk was pasteurized at 72°C for 15 second then cooled to 37 °C. The pasteurized milk divided into three equal portions. Three treatments were carried out as follows: First treatment is the control cheese samples (A) in which cheese was made with conventional yoghurt cultures. In the second and third treatments cheese was made with %100 of probiotics culture (B), and combination of 50% conventional and 50% probiotics culture (C), were added respectively to the milk cheese. Then cheese was made and stored at refrigerator (4°C) for 14 days. Physicochemical and sensory evaluations were done for the cheese samples at day zero, 7, and 14 days, intervals. The cheese statistical analysis showed that probiotics significantly ($P< 0.05$) affected the chemical composition of the cheese. The results also showed that crude protein, acidity, and ash were significantly ($P< 0.05$) affected by levels of probiotics, while fat, total solids and salt showed no significant difference ($P>0.05$). However, the storage period had significant difference ($P<0.05$) on the protein, fat, total solids, acidity, ash content and salt. The sensory characteristics of the cheese samples were found to be affected significantly ($P>0.05$) by the storage period on color, taste, texture, and flavor. Except the saltiness and overall acceptability. Addition of probiotics culture had significant effects on the color, flavor, texture, taste and overall acceptability. It would be concluded that the quality of Sudanese white cheese was relatively improved with the addition of probiotic bacteria (*Lactobacillus acidophilus and Lactobacillus plantarum*).

مستخلص البحث

أجريت هذه الدراسة في معمل الألبان بكلية الإنتاج الحيواني جامعه السودان للعلوم والتكنولوجيا في الفترة من مايو - يونيو (2017) , تهدف الدراسة إلي تقييم تأثير المستويات المختلفه من المعاونات الحيويه (البروبايوتك) (لاكتوباسلس إسيدوفلس و لاكتوباسلس بلنتارم) وفتره التخزين علي الخصائص الكيميائيه والحسيه للجبن البيضاء السودانيه. تم شراء ثلاثون/ لتر من لبن الأبقار الخام من مزرعه الألبان بكليه الأنتاج الحيواني جامعه السودان للعلوم والتكنولوجيا ,تمت معامله اللبن حراريا بالبستره في درجه حراره (72م°) لمده/ دقيقه وتم تبريده إلي (37م°),ثم قسمت إلي ثلاثه مقادير متساويه (10/لتر) للحصول علي ثلاثه معاملات. المعامله الأولي الضابط أضيفت لها بادئ الزبادي التجاري (A), بينما المعامله الثانيه والثالثه أضيفت لها بادئ (البروبايوتك) بنسبه 100% (B), ومزيج من البادئ التجاري 50% وبادئ (البروبايوتك)50% (C) علي التوالي .تم تصنيع الجبن وخزنت في ثلاجه بدرجه حراره (4م°) لمده 14 يوم ,أجري التحليل الفيزيوكيمائي والتقييم الحسي لعينات الجبن في اليوم 7 ,0 , 14 علي التوالي .أجري التحليل الأحصائي للبيانات وأظهرت النتائج أن لفتره التخزين والمعاملات أثر معنوي كبير علي التحليل الكيميائي للعينات حيث وجدت فروق معنويه لأثر المعاملات علي البروتين,الحموضه,الأس الهيدروجيني,والرماد حيث لم تظهر فروق معنويه علي الدهن,الجوامد الصلبه والملح .وكذلك كان لفتره التخزين أثر معنوي كبير علي البروتين,الدهن,الحموضه,الأس الهيدروجيني,الرماد والملح. أوضحت النتائج أن ليس لفتره التخزين أثر معنوي كبير علي التقييم الحسي للعينات من حيث الطعم والقوام والنكهه والملوحه والقبول العام ولكن وجد أثر معنوي علي اللون.بينما كان هناك أثر معنوي لأضافه البروبايوتك علي التقييم الحسي للعينات من حيث اللون والطعم القوام والنكهه والملوحه والقبول العام . وجد ان استخدام المعاونات الحيوية يحسن جودة الجبنه البيضاء السودانيه.

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CHAPTER ONE

INTRODUCTION

It is difficult to separate functional and conventional foods in appearance contrary to conventional foods, functional foods have evidenced physiological benefits, in addition to basic nutritional functions e. g. gut health, they show physiological benefits and can reduce the risk of chronic disease, functional food can provide the needs of the body with the required amount of vitamins, fats, proteins, carbohydrates, etc., needed for its healthy survival (FAO, 2007; Cencic and Chingwaru, 2010).

Probiotics are the most commonly used dietary method of influencing the gut flora composition, a recent definition of probiotics was given as 'a live microbial feed supplement that is beneficial to health (Gibson, 2007). Probiotics are usually used in dairy products, as well as cheese is a good vehicle for these microorganisms, besides the viability of probiotics in cheese, it is important that incorporation of probiotics bacteria should not affect the expected sensory characteristics (flavor, texture, and appearance) of conventional (non-probiotics) cheeses, although several studies have shown probiotics culture didn't considerably affect the sensory quality of cheese, it is thought that their addition might contribute to different flavor and texture characteristic (Karimi *et al*, 2012). Some probiotics mixed culture, e.g. ABT cultures containing (*Lactobacillus acidophilus*, *Bifid bacterium and Streptococcus thermophiles*) have been developed to bring out the preferred flavors in the products in which they are used the

introduction of cultures for direct inoculation of the cheese vat, "direct vat set" (DVS), has allowed culture producers to launch new culture blends, consisting of both *thermophilic* mainly (*S. thermophilus*) and mesophilic strains (Buriti *et al*, 2007). In recent years' cheese is very popular subject of various marketing and research studies, because it is a good alternative for delivery of probiotics into the intestine, fermented milks have long been used as the main vehicles for probiotic strains, the supplementation of cheeses with *probiotics bacteria* represents the aggregation of added value to a product that already has benefits inherent in its composition (Gomes *et al*, 2011; Minervini *et al*, 2012). The ingestion of cheese supplemented with *probiotics bacteria* has been associated with a variety of benefits to human health, such as improvements in the immune system, improvements in oral and intestinal health in the elderly and reinforcement of intestinal immunity (Lollo *et al*, 2012; Albenzio *et al*, 2013a).

Cheese has certain advantages as a carrier of probiotics compared with more acidic fermented dairy products such as yogurt. It creates a buffer against the high acidic environment in the gastrointestinal tract (GIT) and thus creates a more available environment for probiotics survival throughout gastric transit (Karimi *et al* ,2012; Ortakci *et al*, 2012). As already reported by several authors, cheese is a promising food matrix for probiotics, but strain selection and possible process regulations should be carefully evaluated to maximize probiotics cell viability during cheese manufacture and storage as well as to limit possible changes in organoleptic properties it could be said that only a few probiotics cheeses have been successfully developed for the market when compared with yoghurts or fermented milks

because of product quality that can be affected by the addition of some probiotics bacteria (Grattepanche *et al*, 2008).

The Sudanese white cheese is usually characterized as having poor functional therapeutic Therefore, it is crucial to find ways of improving the therapeutic value of the product. The aim of this research was to investigate the possibility of using probiotics culture in the production of white cheese made from cow's milk and to create organoleptic properties of probiotics product.

Objectives of the study:

1. To study the effect of utilizing probiotics bacteria on thephysico-chemicaland sensory characteristics of the Sudanese white Soft cheese.

2.To determine the impact of storage period onthe Physicochemical and sensory evaluation of white cheese.

CHAPTER TWO

LITERATURE REVIEW

2.1. The milk:

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 colostrum's, which carries the mother's antibodies to its young and can reduce the risk of many diseases, milk also can be defined as the normal secretion of the mammary gland of mammals and cannot be a colostrum's or a colostrum's milk (Clarence *et al,* 2004).

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 tons 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). It also contains components that are essential to humans such as proteins, carbohydrates, fat, vitamin D, calcium and phosphorus and it also provides energy (Pauline and Karin, 2006).

2.2. Fermented dairy products:

Fermented dairy products have a long and culturally diverse history. The origins of fermented dairy products can be traced far back to Persian times (8000 BC) in the Middle East when, as it is believed, the art of cheese making was introduced (Rose *et al*, 2002). Fermented milk products originated from near East and subsequently gained popularity in Europe Fermentation process, which occurs in the fermented milk results in conversion of lactose to lactic acid, this acid has preservative effect on milk, the low pH of fermented milk inhibits the growth of undesirable bacteria and pathogenic organisms, the starter cultures used for fermentation of milk convert part of lactose to lactic acid, carbon dioxide, acetic acid, dactyl, acetaldehyde and several other materials (Walstra *et al*., 1999).

2.2.1. Yoghurt:

Yogurt is a fermented product prepared from milk of high solids content some quantity of water is evaporated from milk or skimmed powder is added, this milk of high SNF content is subjected to fermentation by the symbiotic growth of two types of bacteria (*Lactobacillus bulgaricus and Streptococcus thermophilus*), Yoghurt is obtained from pasteurized milk, cream or a mixture of two or more of these products (Mahindru, 2009).

Current trend for using prebiotics and probiotics culture in the manufacturing of fermented milks and yogurt products is in response to consumer expectations for functional or wellness foods, the beneficial effects documented in numerous studies and reviews include; prevention of cancer, reduction in diarrhea associated with travel, antibiotics therapy and rotavirus, improvement of gastrointestinal health, enhancement of immunity

of the host, amelioration of lactose intolerance symptoms, protection from infections caused by food-borne microorganisms and vaccine adjuvant effects (Chandan, 2008).

2.2.2. Cream:

Cream is a fat rich portion separated from whole milk; it contains all milk constituents in varying portions. According to prevention of food adulteration rule (PFA) cream should contain 20-25 % milk fat (Pauline and Karin, 2006).

2.2.3. Butter:

Butter is the concentrated form of milk fat. According to prevention of food adulteration rules (PFA) Butter should contain not less than 80 % of fat, a maximum of 1.5 % curd content and all fat soluble vitamins such as vitamin A,D,E and K and its made by churning cream, sour cream or sour milk (Mahindru, 2009). Pauline and Karin, (2006) added that butter has a limited shelf life and can become mouldy or rancid.

2.2.4. Ghee:

Ghee is produced by removing the last water remnants from butter by heating the butter and letting the water evaporated, or by melting butter and draining the water which separates from the fat (Pauline and Karin, 2006). Fat content is the milk component most quantitatively and qualitatively variable and depend on lactation stage, season, breed, feeding, and genotype (Raynal, *et al* 2008). Ghee is an important milk product which has been extensively used throughout the country for dietary. Ghee is the clarified butter fat prepared either from cream or butter, it is the richest

sources of milk fat among all the dairy products (Ibraheem and Sheeba, 2004). As stated by Mahindru (2009) ghee has a rich and good flavor.

2.3. Cheese:

2.3.1. The origin of cheese:

There is no conclusive evidence indicating where cheese-making originated from, either in Europe, central Asia or the middle East, but the practice had spread within Europe prior to Roman time and it had become a sophisticated enterprise by the time the Roman Empire came into the being (Arvind, 2010). The proposed date for the origin of cheese-making range from around 8000 BC (when sheep were first domesticated) to 300 BC, the people of Greece used cheese from 1000 to 450 BC and the Romans from 775-750 BC (Clarence *et al*, 2004). Approximately one third of the world's milk production is used in cheese manufacturing (Farkye, 2004).

Cheese is one of the most diffused vehicles to hold efficiently *probiotics bacteria*, and thus introduce them into the human diet (Saxelin *et al*, 2003). For a long time, the cow's milk cheeses were mostly studied for the incorporation of probiotics (Bergamini *et al*. 2010; Gomes *et al*, 2011; Ong *et al., 2007*).

2.3.2The definition of cheese:

Fox (2000) defined cheese as the fresh or ripened product obtained after coagulation and whey separation of milk, cream or partly skimmed milk, buttermilk or a mixture of these products. James (2013) gave a simple definition for cheese as a fresh or ripened product obtained after coagulation and whey separation of milk, cream or partly skimmed milk, butter milk or a

mixture of these products. Other definition for cheese was given by Shukla,(2010), and Ramakant, (2006) who stated that cheese is a food made from milk usually the milk of cows, buffalo, goats or sheep by coagulation. Due to differences between regions and the specific technology used in the processing of milk, numerous cheeses were developed, such as Cheddar, Gouda and Mozzarella. These products have one thing in common, namely that the LAB is responsible for the acidification (Law and Tamime, 2010).

2.3.3.Nutritional value of cheese:

Cheese is a rich source of essential nutrients such as proteins, lipids, vitamins and minerals that perform an integral part of a healthy diet (Ash and Wilbey, 2010). The wide variety of cheese all over the world constitutes a growing market for probiotics cheese with potential advantages and is a valuable alternative to the dairy industry (Cruz, *et al*., 2009). Many reduce fat varieties of cheeses are available for individuals monitoring or reducing fat in their diet, also Individuals can include cheese in a fat reduced diet (McBean, 2002). Because of their low lactose, most cheeses are well for individuals.Who have difficulty digesting lactose (lactose mal digesters) (Miller *et al*, 2000).

Moreover, because cheese is high in calcium, it is inclusion in the diet, which reduces the risk for osteoporosis. In addition, cheese in moderation, is includedin the diet designed to reduce the risk of hypertension, also reduce factors for heart disease (McBean, 2002). Food value of cheese is well known and cheese is sometimes called as solid milk. A kilogram of cheese has practically twice the food value of equal quantity of meat. As the manufacturing of cheese is based on break down of milk

protein, it is easily digestible when taken with reasonable quantity of carbohydrates. It is potential source of milk proteins, calcium, phosphorus and fat soluble vitamins (A, D, E, and K) (Subhasish and Subhash, 2006; and Walstra *et al*, 2006). In general cheese supplies a great deal of calcium, protein and phosphorus. A30-gram serving of cheddar cheese contains about 7 grams of protein and 200 milligrams of calcium, nutritionally, cheese is essentially concentrated milk; it takes about 200 grams of milk to provide that much protein and 150 grams to equal the calcium, some studies claim to show that cheese can help to prevent tooth from decaying (Arvind, 2010).

McBean (2002) reviewed cheeses nutritional contribution to the diet, many reduce fat varieties of cheeses are available for individuals monitoring or reducing fat in their diet, also individuals can include cheese in a fat reduced diet. He also reported that certain cheeses have been demonstrated to reduce the risk of dental caries, he added that cheeses have an anti-carcinogenic effect, so consuming cheese may stimulate the flow of saliva, which has caries reducing properties, in addition to that cheese protein has been demonstrated to neutralize plaque acids, which are produced during the fermentation of sugars and starches by plaque bacteria, and cheese appears to prevent acid demineralization and enhance demineralization of tooth and reduce tooth decay.

2.3.4. Cheese storage:

Natural cheese should be stored at low temperatures to ensure good quality, a high temperature leads to evaporation of moisture, which growth of unwanted moulds and taint producing bacteria and other faults, a very low temperature also leads to moulds growth because of the relatively high

humidity usually associated with it and may result in a damaged texture the suitable storage temperature for cheese may be 5-10C° (Ramakant, 2006).

2.3.5. Cheese ripening:

The cheese ripening (maturation) is a complex process involving a range of microbiological and biochemical reactions, microorganisms are present in cheese throughout ripening and contribute to the maturation process either directly through their metabolic activity or indirectly through the release of enzymes into the cheese matrix through autolysis (Fox *et al*, 2004). In fact, cheese ripening is a complex procedure that is accompanied by pH variation, breakdown of proteins, slow accumulation of amino acids and lipid degradation (Farahani, *et al* 2014; Gonzalez-Martín *et al*, 2017).

During the ripening of cheese, three major biochemical events glycolysis, lipolysis, and proteolysis occur each of them is involved in flavor formation, the ripening process varies depending upon the type of cheese, During ripening chemical and enzymatic reactions occur that result in the development of flavor and changes to the body texture and physical properties of the cheese, cheeses ripened into distinct varieties partly because they are made physically different by the technology in the cheese plant and partly because they are made with different microbial cultures,
after the cheese curd has been formed, salted, pressed and placed in the maturation area, its microflora tarts transforming the bland product of the fermentation stage into a cheese whose flavor, texture and appearance are largely dependent on the microorganisms present within the curd mass or on its surface. Some of these microorganisms will have been added deliberately as the starter culture, as a ripening blue or white mould culture, or as a

surface smear of bacteria and yeasts. Others, mainly non-starter lactic acid bacteria (NSLAB) (*lactobacilli and pediococci*) gain access to the cheese from the milk or from the factory environment or added as adjunct culture and contribute to ripening from within the cheese through their biomass (enzymes and substrates) and their metabolism (Law and Tamime,2010).

2.4. Sudanese white soft cheese:

Cheese making is the man preservation method for milk in rural areas of Sudan particularly during rainy seasons when plenty of milk is available (El-Owni and Hamid, 2008).The traditional method of production involves rennting, curd formation, fermentation and final preparation for market (Ibrahim, 2003; El-owni, and Hamid, 2007). Soft white cheese locally known as Jibna-Beyda is the major type of cheese in Sudan, beside Mudaffara, Mozzarella and Gouda which are introduced recently, However, production of processed cheese has also been tried (Nour- El-Daim and El-Zubeir, 2007). For preservation and contribution to flavor, Salt controls microbial, enzyme activity, biochemical changes during ripening and development of flavor and aroma of cheese (Guinee, 2004). Cheese plays an important role in the Sudanese diet, and many people eat a certain amount of cheese with at least twice per week in one of their meals, most of the cheese is consumed either directly or with bread (Dhuol, and Hamid, 2013).

2.4.1. Chemical composition of the Sudanese white soft cheese:

Jibna-Beyda has mean total solids, fat, crude protein and ash of 52.77 , 22.8, 22.50 and 4.87 %, respectively (El-Owni and Hamid, 2007). However, Abdel Razig and Babiker, (2009) reported values of (49.48-53.32), (20.10-22.91), (20.08-23.83) and (2.03-3.53^0), for during the storage of the

Sudanese white cheese at room temperature changes in the chemical composition can occur.

In Sudan different investigators reported variable results concerning the chemical composition of the Sudanese white cheese. Many investigators reported that the white cheese may contain the following chemical composition: fat (13-37%), protein (12.82-22.6), moisture (38.2- 64.21%), ash (1.33 - 4.82), titratable acidity (1.33 -1.60%) and pH (4.4 - 6.3%) depending on the ingredients used in the manufacturing of cheese (Kwak *et al*.,2002; Shirashoji *et al*., 2006; Kapoor *et al*., 2007; and Pinto *et al*., 2007).

Ibrahim, (2003) examined thirty samples of the Sudanese white cheese and he showed that their average content of moisture, salt, pH value and titratable acidities were 44.2, 4.3, 4.6 and 2.3% respectively. Nuser (2001) studied the chemical composition of fresh white cheese and found that the fat content was 25.13%, protein 23.26 %, total solids 48.47 %, ash 3.5 % and titratable acidity was 0.66 %, the average chemical composition of Sudanese white cheese as stated by Sulieman *et al*., (2005) were 50.31% total solids, 49.49% moisture, 20.12 % protein, 22.27 % fat, 4.76 % salt, 5.57% ash, 1.64 % lactose, 4.85 % pH, 1.85 titratable acidity, 1.70 (0.1 N ml NaOH/100 g cheese) volatile fatty acids, 10.02 mg/100 g and acetaldehyde, 30.89 mg/100 g.

2.4.2. Packaging of the Sudanese white soft cheese:

The packaging system ought to be considered as an important stage of the processing of probiotics dairy foods and should be taken into consideration in order to improve the stability of probiotics bacteria in foods. In general, probiotics dairy foods, like cheese, are packaged in plastics films

which have different levels of permeability to oxygen this becomes a problem, because of the strain-dependence, as most members of this microbial group are sensitive to oxygen, due to anaerobic metabolism (Robertson, 2006). Ibrahim, (2003) and Osman,(2005) stated that the essential dairy products including cheese must be safe, acceptable and meet consumer's satisfaction the packaging process undertakes several basic roles such as preventing microbial and chemical quality deterioration and enhancing the handling and marketing for packaged products, now days, food packaging not only targets convenience and protection properties but also presents many other applications such as extending the shelf life and storage of food products.

Han, (2005) found that Plastic containers are now widely used for backing of cheese in Sudan. Abdalla, and Mohamed,(2009) investigated the effect of vacuum packaging on chemical composition and sensory properties of white soft cheese and found that sensory properties are gradually improved, However, vacuum packaging is currently not feasible in rural areas of Sudan where the majority of cheese is produced in a laboratory trial. Nour El Diam and El Zubeir (2007) reported that glass packaging was more acceptable compared to plastic packaging (70% and 30%, respectively).

2.5. Cheese microorganisms

2.5.1. Yeasts:

Yeasts are micro-organisms that can ferment sugars into alcohol, gas and other substances, they are about 5-10 times larger than bacteria. Yeasts usually grow in an acid environment; they need oxygen and they can withstand rather high concentrations of acids in dairy products, yeasts are

usually found in soured products like sour milk, buttermilk, sour whey and on the surface of the cheese, when present in large numbers they produce gas and they cause undesirable off flavors of the product (Ibraheem and Sheeba, 2004).

2.5.2. Moulds:

Moulds are string-like micro-organisms to develop they need atmospheric oxygen and they thrive best in humid and acid conditions, moulds multiply by forming spores their mobility to makes them an important source of infection for some soft cheeses moulds are essential for ripening in general, moulds are harmless but some produce poisonous toxins (mycotoxins) such as aflatoxin (Pauline, and Karin, 2006).

2.6.LAB as starter-cultures in cheese processing:

Cheese-making is based on application of LAB in the form of defined or undefined starter cultures that are expected to cause a rapid acidification of milk through the production of lactic acid, with the consequent decrease pH, thus affecting a number of aspects of the cheese manufacturing process and ultimately cheese composition and quality (Briggiler-Marco *etal*, 2007).

Today, backslapping is still used to produce many artisanal raw-milk cheeses, namely those bearing the PDO (Protected Designation of Origin) status, which are considered to be an important source of LAB genetic diversity, as well as being crucial from an economic and even ecologic point of view, since production of said cheeses (usually processed on a small-scale)contributes to local employment and maintains people functioning as “guardians of local environment” in regions that otherwise would be

deserted the starter-culture applied in this, so-called, natural fermentation, is usually a poorly known microflora mix that although having a predominance of LAB, may also contain non-LAB microorganisms, and its microbial diversity and load is usually variable over time (Bernardeau *et al*, 2008).

Depending on geographical region, where a few may show particular interesting technological features that upon optimization may have industrial applications, For example, because wild strains need to withstand the competition of other microorganisms to survive in their hostile natural environment, they often produce antimicrobials substances called bacteriocins (Ayad et al., 2002). Which are natural antibacterial proteins that can be incorporated directly into fermented foods such as (food–grade) or indirectly as starter culture (Bernardeau *et al*., 2008).

Inhibition of spoilage and harmful microflora by addition of probiotics could be attributed to the nutrient competition among the microorganisms and to the production of some antimicrobial compounds including lactic acid, acetic acid, hydrogen peroxide and some bacteriocins by lactic acid bacteria (Aljewicz and Cichosz, 2015). Although nisin is today the only bacteriocin that reached commercial status, Antilisterial activity of LAB isolates from a traditional cheese Thus, the cheese industry in looking for new types of LAB starter-cultures bearing several properties cultures that increase microbial safety or offer one or more organoleptic, technological, nutritional (enzymes, or polyunsaturated fatty acids - PUFAs) or health advantages such as probiotic properties, starter cultures with increased resistance tobacteriophage, (recall that high product loss, especially in cheese manufacturing, is often16 (*Lactic Acid Bacteria* – R and

D) for Food, Health and Livestock Purposesassociated with bacteriophages (Parente and Cogan, 2004).

2.6.1. *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 around 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 strains are commercially used in many dairy products, sometimes together with (*Streptococcus thermophiles),* and (*lactobacillus delbrueckii subsp bulgaricus),* in the production of *acidophilus*- type yoghurt (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).

Lactobacillus strain, whereas the safety should be proven in animal models (FAO/WHO, 2002; Vesterlund *et al*., 2007; Kõll*et al*., 2010). Next, pilot clinical trials on healthy volunteers are needed, to exclude probiotic administration having adverse effects on gut health and biochemical and cellular indices of the blood, reflecting the proper functions of human organs (Reid, 2005; Rijkers *et al*., 2010).

Also *Lactobacillus* strains are very important because they are recognized for their probiotics activity as well as their fermentative ability (Mirzaei and Barzgari, 2012; Hashemi, *et al*., 2014). Further, only after these procedures should the expression of the functional properties of the straineither by improving some physiological functions (e.g.,antimicrobial, metabolic, immunogenic, anti-oxidative) of the host or by reducedthe risk of some diseases after consumption of the probiotics product be tested in large groups of volunteers several studies have found an inverse relationship between the intake of low-fat dairy products and the incidence of cardiovascular diseases (CVD), preclinicalatherosclerosis, and cardiovascular risk factors in middle-age and older age persons (Engberink *et al*., 2009; Levitan *et al*., 2009;Toledo *et al*., 2009).

However, few safety assessments have been directed toward the control of biomarkers of host basic metabolism, particularly carbohydrates, lipids, and AA turnover, after administration of a dairy probiotics furthere more, it has not been elucidated how the addition of a probiotics strain to a full-fat dairy product affects gut functionality indices of the host here, we report on clinical probiotics food intervention trials in adults and the elderly in which the toiler-ability and safety of a cheese containing a probiotics*L. Plantarum,* some authors suggest that probiotics culture could beneficially

influence the absorption of dietary fat and cholesterol in the small intestine (Agerholm-Larsen *et al*., 2000; Raff *et al*., 2008).

2.7. Probiotics:

The application and development of functional foods are interesting presently. Several food products could be used to improve the health and well-being of the consumers, for commercial applications, probiotics *lactic acid bacteria* are the most favorite and popular *bacteria* applied in food products (Wedajo, 2015). Probiotics can be defined as a food product that contains a sufficient number of viable microorganisms to alter the microflora of the host and has the potential for beneficial health effects, also, it can be defined as live microorganisms that when administered in adequate amounts confer a health benefit on the host (Suresh *et al*., 2013).

Probiotics are defined as 'live microorganisms that, when administered in adequate amounts, confer a health benefit on the host (FAO/WHO, 2002). Probiotics are live bacteria that may confer a health benefit on the host, more than 20 years ago, 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 live microorganisms, which, when consumed in appropriate amounts in the food, confer a health benefit on the host (FAO/WHO, 2001). Their identity, safety, and health claims have attracted a large amount of attention from different public and regulatory organizations. The putative probiotic strain should be accurately characterized and identified (Vankerckhoven *et al*., 2008). Probiotics food is defined as a processed product which contains viable

probiotic microorganisms in a suitable matrix and in sufficient concentration (Saxelin, *et al.*,2003).

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).In the scientific literature, populations of 106e107CFU/g in the final product are established as therapeutic quantities of probiotics culture in processed foods (Talwalkar, *et al.*,2004).Countless benefits to health are provided by the ingestion of foods containing probiotics culture, some with scientific proof, and other ones still needing more human studies. The ingestion of cheese supplemented with probiotics bacteria has been associated with a variety of benefits to human health, such as improvements in the immune system (Ibrahim *et al*., 2010).

These bacteria beneficially affect human health by improving the balance of the intestinal microbiota and improving mucosal defenses against pathogens (Boy-lston, *et al*., 2004). Some of the main beneficial effects on health related to probiotics consumption is antimicrobial activity, prevention and treatment of diarrheas, relief in the symptoms resulting from lactose intolerance, antimutagenic and anticarcinogenic activities, stimulation of the immunological system, improvement of urogenital health, relief of constipation, and optimization of vaccine effects (Bomba, *et al*., 2002; Shah, 2007).

Probiotics bacteria have been recommended for the treatment of atopic dermatitis, necrotizing enter colitis, pseudomembranous colitis, chronic liver disease, allergic disease and food allergy (Boyle and Tang, 2006; and Thomas, 2008). Also, probiotics bacteria can be used to treat

irritable bowel syndrome (Santosa, *et al.*,2006). Probiotics reduce serum cholesterol (Shah, 2007). Hence, the number of available products and the consumer's familiarity with the 'probiotic' concept has been increasing, and, as a consequence, the research into these products has also been increasing. More than 600 food products launched by the dairy industry in (2006) used the term probiotic (Sveje, 2007).

2.7.1. Lactobacillus Plantarum:

L. Plantarun is a Gram-positive-aero tolerant bacterium that grows at 15 c˚ (59 F˚) but not at 45˚C (113 F˚) and produces both isomers of lactic acid (Juana *et al*., 2008). *Lactobacillus plantarum* I91 neither contributed to acid production during cheese manufacture and ripening nor caused changes to the gross composition of the cheeses, which is an important technological feature to prevent deviations from standard cheese-making and overall quality of the product (Crow *et al*.,2002). 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 hippo campal brain derived neurotrophic factor (Kleerebezem and Bokhorst 2003).

CHAPTER THREE

MATERIALS AND METHOD

This study was conducted during the period May-June (2017)at the Department of Dairy Science and Technology, College of Sudan Animal Production Science and technology, Sudan University of Science and Technology. Three treatments were carried out in this study and three level of storage period were used.

3.1. Materials:

3.1.1. Source of probiotic and milk:

Probiotics capsules (*Lactobacillus acidophilus and Lactobacillus plantarum, Gmbh 82515 Wolfratshusen Germany*), from the pharmacy at local market. Thirty liters (30 liters) of cow's milk were purchased from the Dairy Farm, College of Animal Production Science and Technology, Sudan University of Science and Technology, Hillah kuku.

3.1.2. Source of rennet and salt:

Powder animal rennet was obtained from Char-Hansen's Laboratories (Copenhagen, Denmark). Salt from the local market.

3.1.3. Source of startercultures:

A commercialyoghurt utilizing as starter cultures, composed of (*S. thermophilus and L. bulgaricus*) obtained from local market.

3.1.4. Probiotics culture preparation:

One liter of skim milk was sterilized at (85°C) for 60 minutesthen cooled at(37_38°C) then inoculated with the probiotics at the rate of 2% and incubated at (21°C) until coagulation of milk.

3.1.5. The cultures:

Commercial yoghurt cultures, and probiotics culture were added at the rate of 2%of the milk used for Cheese making.

3.2. Methods

3.2.1. Cheese manufacturing:

Cheese manufactured according to the method described by Ibrahim, (2003) and Ahmed and khalifa, (1989), with some modifications. The thirty liters of fresh clean cow's milk were heated in a water bath to (72°C) at15 scond and cooled to (37°C) after divided in to three equal portion (10 liters), probiotics culture was added at the levels of 1.0 and 0.5 to the milk respectively,while control sample (A), had no was added probiotics culture, but 100% conventional yoghurt culture was added. The second (B),added 100% of probiotics culture *(L. acidophilus and L. plantarum)*, the third (C), added both50% conventional yoghurt cultures and 50% probiotics culture (*L.acidophilus*and *L.plantarum*).The milk was stirred gently for 15 minutes. Rennet powder (1 gram/50 liters) was added to milk, stirred for 10 minutes and then left until coagulation occurred. The curd was cut in to small cubes (5x5x5cm). After draining, salted at 2% (w/v) was mixed with the curd according to whom. The curd was poured into clean wooden molds lined

with cheese cloth and pressed overnight. The manufactured cheese samples were stored into triplicate sterile plastic buckets(capacity 150 grams) and stored at refrigerator (4°C) for 14 days. Physicochemical and sensory evaluations of cheese samples were carried out at zero day, 7, and 14days' intervals.

3.2.2. Physico-chemical analysis of milk and cheese:

The chemical composition of the milk was determined after heat treatment of milk (pasteurization).

3.2.2.1. Total solids content:

Total solids content was determined according to the modified methods of AOAC,(2009). Tow grams of cheese (three ml milk) sample were placed in a clean dried flat-bottomed aluminum dish. The weight of sample and the dish were recorded and the dishes were heated on steam bath for 10-15 minutes and placed to air oven at 100°C for three hours, the dishes were transferred to dissector to cool and weighted. Heating, cooling and weighting were repeated several times until the difference between the successive weighting was less than 0.5mg. the total solids were calculated as follows:

Total solids% =W1/W0X100

Where:

W1=weight of samples after drying. W0=Weight of samples before drying.

3.2.2.2. Fat content:

Fat content was determined by Gerber method according to AOAC,(2009) as follows: Ten ml of sulfuric acid (density 1.815gm/ml at 20°C) were poured in to a clean Gerber tube followed by addition of 3 gram minced cheese (10.94ml milk) sample. The tubes were then thoroughly mixed till no white particles were seen, centrifuged at 1100 revolution per minutes (rpm) and transferred to water bath at 65c for three minutes. The column of the fat was then recorded immediately.

3.2.2.3. Crude protein:

The protein content was determined by kjeldahl methods according to AOAC (2009). In kjeldahl flask 3gram cheese (10ml of milk) were placed. Two kjeldahl tubes ,1gm NaSo4 and equivalent of (0.1mg Hg) were added twenty-five milliliters of concentrated sulfuric acid (density of 1.86mg/ml at 20°C) were added to the flask. The mixture was then digested until a clean solution was obtained (2.5hours) and the flask were removed and left to cool. The digested sample was poured into a volumetric flask (100ml) and diluted to 100ml with distilled water. The distillate was received in a conical flask containing 25ml of2% boric acid plus 3 drops of indicator (bromocerol green plus methyl red)The distillation was continued until the volume in the flask was 75ml. the flask was removed from the distillatory. The distillatory was then was then titrated against 0.1N HCL until the end point was obtained (red color). protein content was calculated as follows:

Nitrogen (%) =Tx0.1x0.014x20 x100/ Weight of sample

Protein (%) = Nitrogen (%)x6.38

Where T= Titration figure

0.1= Normality of HCL

0.014= Atomic weight of nitrogen 2/1000

20 = Dilution factors3.4.7. Salt contents determination:

3.2.2.4. Salt contents:

Salt contents of the cheese samples and their whey were determined by titration according to (Breen and Price, 1961). For the determination 10 grams of cheese and 10 ml of whey were weighed each in a conical flask. Fifty ml of warm distilled water at 50 – 60°C were added to each flask and the contents stirred until a homogenous suspension was obtained. The suspension of each sample was transferred to a 250 ml graduated cylinder, diluted to 250 ml with distilled water, mixed thoroughly and allowed to stand for 5 to 10 minutes. When the suspended cheese was settled out, to a 25 ml aliquot of the supernatant of each sample 2 ml of 2% solution of potassium chromate in distilled water was added, the mixture was then titrated with 0.01711 N solution of sliver nitrate, the first discernible color change due to the red color of precipitated sliver chromate was taken to be the end point, Knowing that each ml of sliver nitrate solution is equivalent to 0.1% salt, the percentage of salt was then calculated.

3.2.2.5. Ash:

The ash content was determined according to AOAC (2009). Two grams of cheese and(10ml of milk) were weighed in to a suitable clean and dry crucible and evaporated to dryness on steam bath, the crucible were

placed in muffle furnace at 550 °C for 1.5 –2 hrs., and cooled in desiccators and weighed. The ash content was calculated as following:

Ash% = W1/W0 × 100

Where: W1: weigh of ash W0: weigh of sample

3.2.2.6. Titratable acidity:

Titratable acidity was determined by according to AOAC (2009), Ten grams of cheese (10mlof milk) were weighed and placed in a conical flask and distilled water at 40°C was added until the volume was105ml. the sample was then vigorously agitated and filtered through filter paper (whatman No.41). Twenty-five milliliter of the filtrate were pitted into a porcelain dish and 5 drops of phenolphthalein indicator were added, The sample was titrated against 0.1 Na OH till a faint pink color that lasted for three seconds was obtained. The titration was divided by ten to get the percent lactic acid, the acidity for cheese and milk was calculated as follows:

Titratable acidity (%lactic acid) = T× 4 / W

Where:

T: titration figure W: weight of sample

3.2.3. Sensory characteristics:

The sensory characteristics of the cheese samples were judge by 10 untrained panelists for color, flavor, texture, taste, saltiness, and over all acceptability by using sensory evaluation sheet (Appendix) according to (Larmond 1987).

3.2.4. Statistical analysis:

Statistical analysis was done by using SPSS (2014) version 20 General linear model was used for data analysis (Factorial design). were used to estimate the effect of probiotics culture (*L. acidophilus and L. plantarum*), sensory characteristics, storage period, and interaction between them on the chemical composition of the cow's milk white cheese. Least significance differences (LSD) was used for mean separation between the treatments. The level of significance ($P< 0.05$) was used in this study.

CHAPTER FOURE

RESULTS

Chemical compositions of the pasteurized milk used in the study were protein 3.72%, fat 3.5%, Acidity.17%,T.S 10%.

4.1.Effect of different levels of probiotics culture on the chemical characteristics of Sudanese white cheese:

The effects of different levels of probiotics cultureon thechemical composition of white cheese were presented in (table 1). The crude protein of the cheese samples decreased significantly (P< 0.05) with increase in levels of probiotics culture, the highest crud protein (22.45±.06%) was in control cheese (A)while the lowest one (16.74±.06%) was recorded in the cheese samples (C) with 50% conventional culture and 50%probioticsculture (table 1).

The result illustrated that the fat content of the cheese sample was significantly (P< 0.05) different in all treatments, the highest fat(27.08±.19%) was in the cheese control (A), while the lowest one(26.43±.19%) was found in the cheese samples (C) with50% conventional culture, and 50%probiotics culture (table1).

The titratable acidity of the cheese samples was significantly (P<0.05) affected inall treatments, The highest acidity (1.02±.03%) was secured in the cheese samples(B),with 100% of probiotics culture while the lowest one

(0.95±.03%) was secured by cheese samples (C) with 50% conventional, and 50%probiotics culture(table 1).

The data showed that the highest total solids (39.47±.187%) was in the cheese samples (C) with 50% conventional, and 50% probiotics culture, while the lowest one(35.7±.187%) was recorded in the cheese samples (B) with 100% probiotics culture (table 1).

Ash content of the cheese samples (table 1) were significantly different($P<0.05$). The highest ash content (5.22±.07%) was recorded in the control cheese samples (A), while the lowest one (4.54±.07%) was found in the cheese samples(C) with 50% conventional, and 50% probiotics culture.

The result indicated that there was no significance difference ($P<0.05$) in the salt contents among all treatments. The highest salt (2.23±.041%) was in the cheese samples (C), with50% conventional, and 50% probiotics culture (table1).

4.2. Effect of storage period on the chemical composition of whitecheese:

Result in (table 2) illustrated the effect of storage period on the chemical composition of white cheese. The results revealed that the crude protein of the cheese samples significantly ($P< 0.05$) decreased from (21.08±.06%) at day zero to (18.67±.06%) up to the day14.

The fat content of the cow's milk cheese was affected significantly ($P<0.05$) by the storage period (table2). The highest fat content (27.08±.06%) was found at day zero while the lowest one (25.833±.06%) was at day 7.

Table (1):Effect of different levels of probiotics culture on chemical characteristics of white cheese:

Treatments	Chemical composition%					
	Protein	Fat	Acidity	T.s	Ash	Salt
A	22.45±.06[a]	27.08±.19[a]	0.98±.03[b]	35.12±.187[b]	5.22±.07[a]	2.22±.041
B	20.31±.06[b]	26.89±.19[b]	1.02±.03[a]	35.7±.187[c]	4.90±.07[b]	2.20±.041
C	16.74±.06[c]	26.43±.19[c]	0.95±.03[c]	39.47±.187[a]	4.54±.07[c]	2.23±.041
Sig	**	**	**	**	**	N.S

A = control cheese with100% conventional culture
B = cheese with 100% probiotics culture.
C = cheese with50 % probiotics culture and 50% conventional culture
Sig=significant value (P<0.05).
**=highly significant.
N. S=not significant.

It was clear (table 2) that the titratable acidity of the cheese samples increased significantly (P<0.05) as storage period progressed. It was increased from (0.85±.03%) at day zero to (0.97±.03%) at day14.

The data in (table 2) showed that the total solids content of the cheese was significantly (P<0.05) increased from (35.54±.187%) at day zero to (38.13±.187%) at day 14.The ash content of cheese samples in this study was affected significantly (P<0.05) by the storage period. It was decreased from (5.65±.07%) at day zero to (4.51±.07%) at day14(table2).

The results of the study demonstrated that thesalt content in the cheese samples(Table 2) increased significantly (P<0.05) as the storage period progressed. It was increased from (2.01±.041%) at day zero to (2.29±.041%) at day1.

4.3. Effect of different levels of probiotics culture and storage period on the chemical composition of the Sudanese white cheese:

The different levels of probiotics culture and storage period had significant (p<0.05) effect on the crude protein content of the cheese samples (Table 3) the highest protein content (23.85± .097%) was in the control cheese (A) at day zero, while the lowest one (15.216±0.097%) was in the cheese samples (C) with 50% conventional, and 50% probiotics culture at day14.

Table (2): The Effect of the storage period on the chemical composition of white cheese:

Storage/ Days.	Chemical composition %					
	Protein	Fat	Acidity	T.s	Ash	Salt
Day 0	21.08±.06[a]	27.08±.06[a]	0.85±.03[c]	35.54±.19[c]	5.65±.07[a]	2.01±.041[c]
Day 7	20.31±.06[b]	25.84±.06[c]	0.91±.03[b]	36.62±.19[b]	4.53±.07[b]	2.29±.041[a]
Day14	18.67±.06[c]	27.0±.06[b]	0.97±.03[a]	38.13±.19[a]	4.51±.07[c]	2.27±.041[b]
Sig	**	**	**	**	**	**

Sig=significant value($P<0.05$)

**=highly significant.

The results of the fat contentof the cheese samples (Table 4) shows thatit was significant ($p<0.05$) affected by the levels of probiotics culture and storage period. The highest fat content (27.77±.321%) was in the cheese samples (C), with 50% conventional culture, and 50% probiotics culture at day zero, while the lowest value (25.778±.321%) was in control cheese (A) at day7.

The results of the study indicated that in (table 5) addition of different levels of probiotics culture, and the storage period had significant ($p<0.05$) effect on the titratable acidity of the cheese samples, the highest titratable acidity (1.769±0.042%) was inthe cheese samples (B), with 100% probiotics culture at day0. The lowest value (.733± 0.042%) was in the cheese samples (C), with 50% conventional, and 50% probiotics culture at day 14.

The results revealed that the different levels of probiotics culture and storage period had significant ($p<0.05$) effect on the total solids in cheese samples, in (table 6). The highest value (43.20±.323%) was in the cheese samples (C), with 50% conventional and 50%probiotics culture at day14, the lowest value (34.37±.323%) was for the cheese samples (C),with 50% conventional and 50% probiotics culture at day0.

The different levels of probiotics culture and storage period had significant ($p<0.05$) effect on ash content in the cheese samples (Table 7). The highest ash content (5.873± .117%) was in the control cheese (A), at day0, the lowest value (4.14± 0.117%) was in cheese samples (B), with 100% probiotics culture at day 14.

Table (3): Effect of different levels of probioticsculture and storage period on protein content of the white cheese:

Treatments	Storage period			
	Day1	Day7	Day14	Sig
A	23.842±.097[a]	22.759±.097[a]	20.744±.097[a]	**
B	20.992±.097[b]	20.152±.097[b]	20.041±.097[b]	
C	18.400±.097[c]	16.579±.097[c]	15.216±.097[c]	

A = control cheese with100% conventional culture.
B = cheese with 100%probiotics culture.
C = cheese with 50 % probiotics culture and 50% conventional cultures.
**=highly significant.
Sig=significant value (P<0.05).

Table (4): Effect of the different levels of probioticsculture and storage period on the fatcontent (%) of the whit cheese:

Treatment	Storage period			
	Day1	Day7	Day14	Sig
A	$26.96\pm.321^{b}$	$25.778\pm.321^{c}$	$27.500\pm.321^{a}$	**
B	$26.944\pm.321^{c}$	$26.278\pm.321^{a}$	$27.444\pm.321^{b}$	
C	$27.77\pm.321^{a}$	$26.056\pm.321^{b}$	$26.056\pm.321^{c}$	
Sig	**			

A= control cheese with 100% conventional culture.
B = cheese with 100% probiotics culture.
C = cheese with50 % probiotics culture and 50% conventional cultures
**=highly significant. Sig=significant value ($P<0.05$).

Table (5): The Effect of the storage period and different levelsprobiotics culture on the acidity (%) of white cheese.

Treatment	Storage period			
	Day1	Day7	Day14	Sig
A	1.618±.042[b]	1.538±.042[a]	.853±.042[b]	**
B	1.769±.042[a]	1.38±.042[b]	.951±.042[a]	
C	1.356±.042[c]	1.253±.042[c]	.733±.042[c]	
Sig	**			

A = control cheese with100% conventional culture.
B = cheese with 100%probiotics culture.
C = cheese with50 % probiotics culture and 50% conventional cultures.
Sig=significant value (P<0.05).
**=highly significant.

Table (6):Effect of different levels of probiotics culture and storage period on the total solids of whit cheese:

Treatments	Storage period			
	Day1	Day7	Day14	Sig
A	34.37±.323[c]	35.59±.323[c]	35.40±.323[c]	**
B	35.40±.323[b]	36.02±.323[b]	35.69±.323[b]	
C	36.86±.323[a]	38.25±.323[a]	43.20±.323[a]	
Sig	**			

A = control cheese with100% conventional culture.
B = cheese with 100% probiotics culture.
C = cheese with50 % probiotics culture and 50% conventional cultures.
Sig=significant value (P<0.05).
N.S =not significant.

The different levels of probiotics culture and storage period had significant ($p<0.05$) effect on the salt content in the cheese samples (table 8). the highest salt value (2.36±.071%) was for the control cheesesamples (A), at day 14, while the lowest one (2.00±.071%) was in the control cheese (A), at day 1.

4.4. The Effect of different levels of the probiotics culture on the sensory characteristics of the white cheese:

Table (9) showed the main effect of different levels of probiotics culture on the sensory characteristics of cow's milk cheese. significant variations ($P<0.05$) were found in the color, taste, texture, and flavor of the cheese samples, however, no significances differences ($P<0.05$) were observed in saltiness and overall acceptability in the cheese samples.

The cheese samples (B), with 100% probiotics culture showed highest color scores (7.23±.463), while the lowest one (6.63±.463) was for the cheese samples (C), with 50 % conventional culture, and 50% probiotics culture.

The best values for the taste (7.15±.491) was in the control cheese samples (A), while the lowest one (6.78±.491) was in the cheese samples (B), with 100 % probiotics culture.

The control cheese samples (A), recorded the highest texture (7.37±.471), while The lowest value (6.49±.471) was in the cheese samples (C), with 50% conventional culture, and 50% probiotics culture.

The cheese samples (B),with 100% probiotics culture showed highest flavor scores (7.0±.48), while the lowest one (6.49±.48) was in the control cheese (A).

4.5. Effect of storage period on the sensory characteristics of white cheese:

The data in (Table 10) indicated that no significantly ($P<0.05$) variations were obtained for the, taste, texture, flavor, saltiness and overall acceptability. of the cheese samples except color. The result in (table10) indicated that significantly ($P<0.05$) affected of the cheese samples during storage period, the highest color scores (7.8±.27) were at day0 and the lowest value (6.49±.72) was at day7.

Table (7)Effect of different levels of probiotics culture and storage period on ash content (%) of the whit cheese:

Treatments	Storage period			
	Day1	Day7	Day14	Sig
A	5.873±.117[a]	4.809±.117[a]	4.972±.117[a]	*
B	5.780±.117[b]	4.57±.117[b]	4.41±.117[b]	
C	5.278±.117[c]	4.196±.117[c]	4.14±.117[c]	
Sig	*			

A = control cheese with100% conventional culture.
B = cheese with 100% probiotics culture
C = cheese with50 % probiotics culture and 50% conventional cultures.
Sig=significant
*= significant

Table (8):Effect of different of probiotics culture and storage period on salt content (%) of the white cheese:

Treatments	Storage period			
	Day1	Day7	Day14	Sig
A	2.00±.071^{c}	2.25±.071^{b}	2.36±.071^{a}	*
B	2.14±.071^{a}	2.32±.071^{a}	2.17±.071^{c}	
C	2.12±.071^{b}	2.21±.071^{c}	2.27±.071^{b}	
Sig	*			

A = control cheese with100% conventional cultures
B = cheese with 100% probiotics culture.
C = cheese with50 % probiotics culture and 50% conventional cultures.
Sig =significant.
*= significant

Table (9): The Effect of the different of probiotics culture on sensory evaluation of white cheese:

Treatments	Sensory Attributes					
	Color	Taste	Texture	Flavor	Saltiness	Overall
A	6.71±.47[b]	7.15±.41[a]	7.37±.48[a]	6.49±.48[c]	3.21±.41	7.21±.39
B	7.23±.47[a]	6.78±.41[c]	7.21±.48[b]	7.0±.48[a]	3.51±.41	7.32±.39
C	6.63±.47[c]	7.0±.41[b]	6.49±.48[c]	6.83±.48[b]	3.82±.41	7.45±.39
Sig	**	**	**	**	N.S	N.S

A = control cheese with100% conventional culture.
B = cheese with 100% probiotics culture.
C = cheese with50 % probiotics culture and 50% conventional culture.
Sig=significant value (P<0.05).
**=highly significant

Table (10): The Effect of storage period on the sensory characteristics of white cheese:

Storage /Days	Sensory Attributes					
	Color	Taste	Texture	Flavor	Saltiness	Overall
Day0	7.80±.27a	6.93±.29	7.37±.28	6.78±.28	3.89±.29	7.67±.23
Day7	6.49±.27c	6.93±.29	7.45±28	6.63±.28	3.23±.29	7.21±.23
Day14	7.00±.27b	7.08±.29	7.37±.28	6.93±.28	3.51±.29	7.21±.23
Sig	**	N.S	N.S	N.S	N.S	N.S

Sig=significant value($P<0.05$).
**=highly significant
N. S=not significant.

CHAPTER FIVE
DISCUSSION

The protein of the cheese samples was affected by different levels of probiotics culture(Table 1). It was decreased with the different levels of probiotics culture, the decreased of protein could be due to proteolysis activitiesas performed by the enzymes in the rennet preparation used to coagulate milk, indigenous milk proteases and bacterial proteases, these results were harmony with those reported by Bezerra *et al*., (2016) who reported that; while proteolysis is primarily conducted by enzymes naturally found in milk, coagulant agents and microbial enzymes produced by lactic acid bacteria. The degradation of casein to amino acids and further metabolism of these amino acids release aroma compounds play a vital role in cheese flavor formation inseveral of production stages.

Fat of the cheese samples decreased with the increasing levels of probiotics culture (Table 1). This could be attributed to the high lipolysis activities of probiotics strain. This was in accordance with Grattepanche *et al*.,(2008). Lipolysis is not influenced by probiotic bacteria, as them enzymes have a lower lipolytic activity, when compared to starters and NSLAB.

The titratable acidity of the cheese samples was increased with probiotics culture, this increased could be explained the activities of probiotics cultureby Glycolysis as ferment lactose to lactic acid which increase the acidity of cheese samples. Glycolysis involves several reactions, mainly associated to lactose degradation (Bezerra *et al*., 2017; McSweeney

2011) who's reported that during cheese making, lactose is rapidly metabolized by the starter culture to lactate.

Total solids of the cheese samples increased with the increasing levels of probiotics culture (Table 1), this could be due to different moisture content in the cured during manufacture. Data in (table 1) showed that ash content decreased with the different levels of probiotics culture the decreased could be due to activation metabolism bacteria during manufacture or could be attributed to absorption of high level of moisture by curd. Salt content of the cheese samples in this study were not affected by the addition of probiotics culture (table 1).

The storage period significantly ($P<0.05$) affected the protein, fat, acidity, ash, and salt, of the cheese samples(Table 2).Protein content of the cheese samples in this study decreased with the storage period may be due to direct result of protein degradation by activities probiotics culture (table2).This was in agreement with Sousa *et al.*,(2001) limited refrigerated shelf life, have as their main event the primary proteolysis, which is performed by the coagulating agents and, to a lesser extent, plasmin, residual coagulants, and enzymes from the starter organisms. The supplementation of cheeses with probiotic bacteria only has a relevant effect on secondary proteolysis, resulting in an increase in the total free amino acid content and the formation of compounds responsible for flavor and aroma, resulting from the catabolism of these amino acids (Cruz *et al.*,2009).

The fat content was highest at day0 and decreased at 7, and14 days. This might be due to the concurrent loss of protein through proteolysis during storage period. The reduction in fat content could, at first glance, seem surprising. However, this is a common process during cheese ripening,

mostly due to lipolysis occurrence (Kondyli*et al*., 2016).These results were in accordance with Nuser, (2001) who found that the fat content was high at the beginning of storage period, then decreased towards the end of storage period degradation of milk fat may also be an important factor in cheese flavor formation. (Collins, and McSweeney, 2003). The triglycerides are degraded to free fatty acids, di- and monoglycerides and glycerol by esterases and lipases. Generally, the esterases that work in water solution both hydrolyse and form ester bonds, and therefore they play a vital role in ester formation in cheese ripening.

Data in (Table 2) showed that acidity tends to increase during storage period. The acidity of the cheese samples increased significantly as the storage period progressed this could be due to the fermentation of lactose into lactic acid by the starter cultures and may be due to the higher content of non-protein nitrogen and vitamins which are needed for fast growing microorganisms.

Total solids of the cheese samples increased during the storage period (Table 2). This increased of total solids could be attributed to decrease in the moisture content as a result of lactic acid developments by probiotics culture which caused curd contraction. Also could be explained byuse of the probiotics culture which caused the higher titratable acidity values; high titratable acidity in a cheese increases the extent of whey separation and results in increased total solids. Similar results were obtained by Abdalla *et al., (*2011), Abdel-Razig and Babiker, (2009), El-Owni and Hamid (2008) who reported that total solids of the Sudanese white soft cheese increased during the storage period.

Ash contents of the cheese samples in this study decreased with the storage period (Table 2). Decreased in ash content may be due to absorption of moisture by the curd in the refrigerators which decrease the concentration of the minerals in the cheese. These results werenot in accordance with those obtained by Abdalla *et al*. (2011); Elowni and Hamid (2008) and and Babiker (2009) who found that the ash contents of a Sudanese white soft cheese increased during the storage period.

Data in (table 2) showed that salt of the cheese sample increased during the storage period. These results could be due to loss of moisture during storage. These results were in harmony with those reported by Abdalla and Hassan (2013) who showed that sodium contents of the Sudanese white cheese decreased with storage period.

The interaction between the storage period and the addition of probiotics culture significantly affected the chemical composition of the cheese samples in all treatments (Tables 3, 4, 5, 6,7, and 8). The highest protein content % was for the control cheese samples with 100% conventional culture at day 0. However, the lowest protein content in the cheese samples with50%conventional, and 50%probiotics culture. This could be due to proteolytic activities which that degraded protein by probiotics culture consequently tends to decrease the protein content % in cheese samples. Albenzio *et al*., (2013) reported that, the probiotics strain produced greater proteolytic activity than conventional strains used for cheese-making. The probiotics bacteria enzymes act in the secondary proteolysis, increasing the total free amino acid content, which contributes decisively to cheese flavors (sweet, bitter or malty) and can be precursors for

the synthesis of other flavors or volatile aroma, resulting in off-flavors (Ardo¨, 2006).

The mean sensory scores of the organoleptic evaluation and acceptability for the different cheese samples are shown in (Table 9). The statistical analysis explained that there were significant differences ($p<0.05$) among the cheese samples in the sensory attributes color, test, texture, and flavor. The nutritional and sensory improvements in probiotics cheeses are related to the wide spectrum of enzymes that are contained in probiotics, catalyze biochemical reactions over the period of cheese storage, and lead to the production and release of different compounds that affect the quality of the final product, especially the texture and flavor (Albenzio *et al*., 2013).

The flavor, texture and taste followed the same trend. The best flavor and taste scores were for the cheese with 50% conventional starter culture,and50% probiotics culture. Improvement in flavor and taste was probably due to the effect of high lactic acid contents which inhibitthe growth of spoilage and contaminate microorganisms, and contribution to the taste and flavor. Degradation of protein into peptides and amino acid, lipolytic fat to fatty acid and volatile fatty acid which mainly contribute to desirable taste and flavor.

The storage period was no significantly ($P>0.05$) affected the sensory characteristics of the cheese samples. (Table 10) except the color of the cheese. These results could be due to deterioration of color of cheese sample during storage period by probiotics culture. Probiotics culture does not tend to strongly modify the sensorial properties of the products to which they are added (Champagne and Gardner, 2005).

CHAPTER SEX

CONCLUTION AND RECOMMENDATION

6.1. Conclusion

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

 The quality of Sudanese white soft cheese was relatively improved with the addition of probiotics bacteria(*L. Acidophilus and L. plantarum).*

 Significant variations were found in the sensory and physcio-chemical characteristics of the cheese with Probiotics.

 Storage period had significant effect on the physio-chemical and sensory characteristics of cheese.

6.2 Recommendations

*Further work will be required for the effectof probioticson the microbial load of the white cheese.

* Production of Probiotics cheese as a new kind of dairy products must be adopted.

***** More studies about evaluation of health benefits of Probiotics cheese.

REFERENCE

Abdalla, I. M and Hassan, A. F. (2011**)**. Minerals composition (Ca, Na, Pb and Sn) of Sudanese white soft cheese. International Journal of Scientific and Research Publications.,3 (5):1-4.

Abdalla, M.O.; El-Owni, O.A and Kheir, S.A.(2011). Effect of storage periodon quality of Sudanese white cheese (Gibna Bayda) manufactured with Solanum dubium. U of K. J. Agric. Sci.,19 (2):202-217.

Abdalla, M.I.; El-Zubier, M.I and Hassan,F.A. (2013).Effect of packaging technique in physicochemical composition of Sudanese white soft Cheese. International Journal of Scientific and Research Publications., 2 (3):11-17.

Abdalla, M.O and Mohamed, S.N.(2009). Effect of storage period on chemical composition and sensory characteristics of vacuum packaged white softcheese. Pak.J.Nut.,8 (2):145-147.

Abdalla, M.O. (1992). Effect of processing conditions on microbiological quality of white pickled cheese. Ph.D. Dissertation, University of Tennessee. Knoxville, U.S.A.

Abdalla, M.O and Abdel- Razig,K.A.(1997). Effect of type of milk on the quality of white soft cheese. U of K.J.Agric, Sci., 5: 147-157.

Abdel-Razig, K. A and Babiker, A. N.(2009). Chemical and microbiological properties of Sudanese white soft cheese made by direct acidification technique. Pak.J. Nut., 8(8):1138-1143.

Agerholm-Larsen, L. M. L.; Bell, G. K.; Grunwald, R. and AstrupA.(2000). The effect of a probiotic milk product on plasma cholesterol: A meta-analysis of short-term intervention studies. Eur. J. Clin. Nutr., 54:856–860.

Ahmed, A.M. (1987). The manufacture and packaging of the Sudanese white cheese. Paper presented at the UNESCO, Regional training course on fermented foods of the Arab world. 1-15 Feb. (1987), Food Research Center, Shambat, Sudan.

Albenzio, M.; Santillo, A.; Caroprese, M.; Ruggieri, D.; Napolitano, F. and Sevi, A. (2013a). Physicochemical properties of Scamorza ewe milk

cheese manufactured with different probiotic cultures. Journal Dairy of Science., 96: 2781–2791.

Ali, M.Z. (1987). Technology of cheese making in the Sudan. Paper presented at the UNESCO regional training course on fermented foods of the Arab world, 1-15 February. Food Research Center, Shambat, Sudan.

Aljewicz, M., and Cichosz, G. (2015). Protective effects of Lactobacillus cultures in Dutch-type cheese-like products. LWT - Food Science and Technology., 63: 52-56.

Alla Gabo, H.I. (1986). Studies on composition and quality of Gibna Beyda. M.Sc. Thesis, University of Khartoum, Sudan.

AOAC.(2009). Official Methods of Analysis of the Association of Official Analytical Chemists, P.O. Box 540, Benjamin Franklin Station, Washington, D.C.

Ardo, Y. (2006). Flavor formation by amino acid catabolism. Biotechnology Advances., 24(2): 238-242.

Arvind, N. S. (2010). Text book of dairy chemist. Discovery Publishing House PVT.LTD. New Delhi. First Published. India.

Ash, A. and Wilbey, A. (2010). The nutritional significance of cheese in the UK diet. International Journal of Dairy Technology., 63: 305-319.

Ayad, E. H. E.; Verheul, A.; Wouters, J.T.M and Smit,G. (2002). Antimicrobial-producing wild Lactococci isolated from artisanal and non-dairy origins. Int. Dairy J., 12: 145-150.

Bergamini, C.; Hynes, E.; Meinardi, C.; Suarez, V.; Quiberoni, A. and Zalazar, C. (2010). Pategrás cheese as a suitable carrier for six probiotics culture. *J Dairy Res.*,77:265–272.

Bernardeau, M.; Vernoux, J. P.; Henri-Dubernet, S.; Guéguen, M. (2008). Safety assessment of dairy microorganisms: The Lactobacillus genus Int J Food Microbiol., 126: 278–285.

Bezerra, T. K. A.; Arcanjo, N. M. O.; Garcia, E. F.; Gomes, A. M. P.; Qeiroga, R. C. R. E. and de Souza, E. L., (2017). Effect of supplementation with probiotic lactic acid bacteria, separately or

combined, on acid and sugar production in goat “coalho” cheese. LWT - Food Science and Technology., 75: 710-718.

Bezerra, T. K. A.; De Araujo, A. R. R.; Do Nascimento, E. S.; De Matos Paz, J. E.; Gadelha, C. A.; Gadelha, T. S. and Madruga, M. S. (2016). Proteolysis in goat “coalho” cheese supplemented with probiotic lactic acid bacteria. Food Chemistry., 196: 359-366.

Bomba, A.; Nemcova´, R.; Mudronova´, D.and Guba, P. (2002). The possibilities of potentiating the efficacy of probiotics. Trends in Food Science and Technology.,13(4): 121-126.

Boyle, R. J.and Tang, M. L. K. (2006). The role of probiotics in the management of allergic disease. Clinical and Experimental Allergy.,36(5): 568-576.

Boylston, T. D.; Vinderola, C. G.; Ghoddusi, H. B. and Reinheimer, J. A.(2004). Incorporation of bifidobacteria into cheeses: challengesand rewards. International Dairy Journal.,14: 375-387.

Briggiler-Marcó, M.; Capra, M. L.; Quiberoni, A.; Vinderola, G.; Reinheimer, J.A.and Hynes, E.(2007). Nonstarter Lactobacillus strains as adjunct cultures for cheese making: in vitrocharacterization and performance in two model cheeses. J Dairy Sci., 90: 4532-4542.

Buriti, F. C. A.; Okazaki, T. Y.; Alegro, J. H. A. and Saad, S. M. I. (2007). Effect of a probiotics mixed culture on texture profile and sensory performance of Minas fresh catabolism in cheese: a review of current knowledge. International Dairy Journal., 13: 841-866.

Cencic, A., and Chingwaru, W. (2010). The role of functional foods, nutraceuticals, and food supplements in intestinal health. Nutrients., 2(6): 611-625. PMid:22254045PMCid:PMC3257668. http://dx.doi.org/10.3390/nu2060611 [Links].

Champagne, C. P. and Gardner, N. J. (2005). Challenges in the addition of probiotic cultures to foods. Critical Review in Food Science and Nutrition., 45(1): 61-84.

Chandan, R.C. (2008). Role of milk and dairy foods in nutrition and health,” Chapter 18. Dairy processing and quality assurance. Chandan, R.C.; Kilara, A.; Shah, N.P.; Wiley Blackwell, Ames, I.A.International Dairy Journal.,32 (8):411-428.

Clarence, H.E.; Wilies, B.C and Harold, M. (2004). Milk and milk products.4th Edition.

Collins, Y.; McSweeney, P. and Wilkinson, M. (2003). Lipolysis and free fatty acidand esterification. International Dairy Journal., 15: 711-718.

Crow, V.; Curry, B.; Christison, M.; Hellier, K.; Holland, R. and Liu, S.Q. (2002).Raw milk flora and NSLAB as adjuncts. Aust J. Dairy Technol. 57: 99-105.

Cruz, A. G. F.; Buriti, C. B. H.; Souza, J. A. F.; Faria, and Saad S. M. I. (2009). Probiotic cheese: Health benefits, technological and stability aspects. Trends Food Sci. Technol., 20:344-354.

Dhuol, K.R.R. and Hamid, O.I.A. (2013). Physicochemical and sensory characteristics of white soft cheese made from different levels of cassava powder (Manihot esculenta). *International J. of Current Research and Academic Review*, vol., 6 (4): 01-12.

El-Owni, O.A.O and Hamid, O.I.A.(2008). Effect of storage period on weight loss, chemical composition, microbiological and sensory characteristics of Sudanese white cheese (Gibna Bayda), Pak. J. Nut.,7 (1): 75-80.

El-Owni, O.A.O and Hamid, O.I.A. (2009). Effect of type of storage container on weight loss, chemical composition, microbiological properties and sensory characteristics of Sudanese white cheese (Gibna Bayda). Aust. J. Basic Appl. Sci., 3(2): 432-439.

Elowni, O.A.O. and Hamid, O.I.A. (2007). Production of white chees (Gibna bayda) in Zalingei area, West Darfur (Sudan*).* Aust., J, Basic Applied Science.,1: 756-762.

Engberink, M. F. M.; Hendriksen, E. G.; Schouten, F. J. A.; van Rooij, A.; Hofman, J. C. M.; Witteman, and J. M. Geleijnse. (2009). Inverse association between dairy intake and hypertension: The Rotterdam Study. Am. J. Clin. Nutr., 89:1877-1883.

Farahani, G.; Ezzatpanah, H., and Abbasi, S. (2014). Characterization of Siahmazgi cheese, an Iranian ewe's milk variety: Assessment of physico-chemical, textural and rheological specifications during ripening. LWT - Food Science and Technology., 58: 335-342.

FAO/WHO, (Food and Agriculture Organization of the United Nations/World HealthOrganization). (2001). Available from Evaluation ofhealth and nutritional properties of probiotics in food includingpowder milk with live lactic acid bacteria. Report of a joint FAO/WHO expert consultation, Co´rdoba, Argentina. ftp://ftp.fao.org/es/esn/food/probioreport_en.pdfJournal of Probioticsand Prebiotics.,2(23): 75-84.

FAO/WHO, (Food and Agriculture Organization of the United Nations/World Health Organization). (2002). Guidelines for the evaluation of probiotics in food. Food and Agriculture Organization of the United Nations and World Health Organization Working Group Report. Accessed July 20, (2011). http://www.who.int/foodsafety/fs_management/en/probiotic_guidelines.pdf.

Farky,N.Y.(2004). Cheese technology. International Journal of Dairy Technology., 57:91-98.

Faye, B. and Konuspayeva, G. (2012). The sustainability challenges to the dairy sector– The growing importance of non-cattle milk production worldwide. International Dairy Journal., 24 (2): 50-56.

FAO. (2007)Food and Agriculture Organization of the United Nations. Authors Report on Functional Foods, Food Quality and Standards Service (AGNS). Retrieved from,http://www.fao.org/ag/agn/agns/files/Functional_FoodsReport_Nov(2007). pdf. [Links].

Fox, P.F.(2000). Ripening of cheddar cheese made from blends of raw and pasteurized milk. International Dairy Journal., 10: 33-44.

Fox, P.F.;Guinee, T.P.; Cogan, T.M. and McSweeney, P.L.H.(2004). Pathogens and foodpoisoning bacteria in cheese. In fundamentals of cheese science,Aspen Publishers, Gaithersburg., 7 (3): 484-503.

Gibson, G. R. (2007). Functional foods: probiotics and prebiotics. Culture.International Dairy Journal.,28: 1-8.

Gismondo, M. R.; Drago, L.and Lombardi,A. (1999).Review of Probiotics available to modify gastrointestinal flora.Int.J antimicrob. Agents., 12(4):287-92.

Grattepanche, F.; Miescher-Schwenninger, S.; Meile, L. and Lacroix, C. (2008). Recent developments in cheese cultures with protective 352 A. Gomes da Cruz et al. / Trends in Food Science and Technology 20 (2009) 344-354 and probiotic functionalities. Dairy Science and Technology., 88(45): 421-444.

Gomes, A.; Braga, S.; Cruz, A.; Cadena, R.; Lollo, P.; Carvalho, C.; Amaya-Farfán, J.; Faria, J. and Bolini, H. (2011). Effect of the inoculation level of *Lactobacillus acidophilus* in probiotics cheese on the physicochemical features and sensory performance compared with commercial cheeses. J Dairy Sci., 94:4777-4786.

Gonzalez-Martín, M. I.; Palacios, V. V.; Revilla, I.; Vivar-Quintana, A. M., and Hernandez-Hierro, J. M. (2017). Discrimination between cheeses made from cow's, ewe's and goat's milk from unsaturated fatty acids and use of the canonical biplot method. Journal of Food Composition and Analysis., 56: 34-40.

Guinee, T.P., (2004). Salting and the role of salting in cheese. Int. J. Daily Technol.,39: 99-1 .

Gursoy, O., and KinikO.,(2010). Incorporation of adjunct cultures of *Enterococcus faecium*, *Lactobacillus paracasei* ssp. *paracasei* and *Bifidobacterium bifidum* into white cheese. J. Food Agric. Environ., 8:107-112.

Han, J.H. (2005). New technologies in food packaging: Overview. In: Han J.H Innovations in food packaging. Academic, London., 42:3-11.

Harding, F. (1999). Milk quality. Official Journal of the European Union., 421(222):1-28.

Hashemi, S. M. B.; Shahidi, F.; Mortazavi, S. A.; Milani, E. and Eshaghi, Z., (2014). Synbiotic potential of Doogh supplemented with free and encapsulated *Lactobacillus plantarum* LS5 and *Helianthus tuberosus* inulin. J. Food Sci. Technol., 15:244-264http://dx.doi.org/10.1007/s13197-014-1511-7.

Hayaloglu, A. A.; Guven, M., and Fox, P. F. (2002). Microbiological, biochemical and technological properties of Turkish White cheese "Beyaz Peynir". International Dairy Journal., 12(8): 635-648. http://dx.doi.org/10.1016/S0958-6946 (02)00055-9 [link].

Holmes, R. P.; Goodman, H. O. and Assimos, D. G. (2001). Contribution of dietary oxalate to urinary oxalate excretion. Kidney Int., 59: 270-276.

Ibraheem, C. K and Sheeba, K. (2004). Milk production and processing. Daya Publishing House. New Delhi, India.

Ibrahim, A. (2003). Studies on some characteristics of Sudanese white cheese. Sudan,J.Vet.Sci. Anim. Husbandry., 12:31-39.

Ibrahim, F.; Ruvio, S.; Granlund, L.; Salminen, S.; Viitanen, M. and Ouwehand, A. (2010). Probiotics and immunosenescence: Cheese as a carrier. FEMS Immunol. Med. Microbiol., 59:53-59.

Ibrabim, A.A. (2003). Effect of processing and storage conditions on the chemical composition and microbial quality of white soft cheese. M.Sc. Thesis, University of Khartoum, Sudan.

James, Y.B. (2013). Hand book of dairy products. Blume Random Exports, New Delhi, India.

Juana, F.; Soo Song, C.; Martínez-Villalueng, E.; Gonzlez, A.; De Mejia, D. and Vidal-Valverde, C. (2008). Immunoreactivity and Amino Acid Content of Fermented Soybean Products. J. Agric. Food Chem., 56 (1): 99-105.

Kapoor, R.; Metzger, L. E.; Biswas, A. C. and Muthukum, M. K.(2007). Effect of natural cheese characteristics on process cheese properties. J. Dairy Sci., 90:1625-1634.

Karimi, R.; Mortazavian, A. M., and Karimi, M. (2012). Incorporation of Lactobacillus casei in Iranian ultrafiltered Feta cheese made by partial replacement of NaCl with KCl. Journal of Dairy Science., 95(8): 4209-4222. http://dx.doi.org/10.3168/jds.2011-4872 [Link]

Khalid, E.A.(1991).The effect of salt concentration on the yield and chemical composition of Sudanese white cheese. M.Sc.Thesis, University of Khartoum, Sudan.

Khateeb, A.H.(1997). An Attempt to standardize the manufacturing procedure Sudanese white cheese.M.Sc. Theis University of Khartoum, Sudan.

Kim, K.S.; IN, Y.N.; Lee, S.Y and Chung, Y.H.(1992). Changes of physiochemical characteristics of natural cheddar cheese during

ripening. Research reports of the rural development administration; Livestock., 34(2):29-32.

Kleerebezem, M. and Boekhorst, J. (2003). Complete genome sequence of lactobacillus plantarum WCFSI. Proceedings of the National Academy of Sciences.,100(4):1990-2005.

Kõll, P. R.; Mändar, I.; Smidt, P.; Hütt, K.; Truusalu, R. H.; Mikelsaar, J.; Shchepetova, K.; Krogh-Andersen, H.; Marcotte, L.; Hammarström, and M. Mikelsaar. (2010). Screening and evaluation of human intestinal lactobacilli for the development of novel gastrointestinal probiotics. Curr. Microbiol., 61:560–566.

Kondyli, E.; Pappa, E. C., and Svarnas, C. (2016). Ripening changes of the chemical composition, proteolysis, volatile fraction and organoleptic characteristics of awhite-brined goat milk cheese. International Dairy Journal., 56(12):115-132.

Kosikowski , F.V.(1982). Cheese and fermented milk Food. Edwards Brothers, Inc., Ann. Arbor., Michigan, USA.

Kur, L.L.A.(1992). Effect of storage on the quality and chemical composition of Sudanese white cheese. M.Sc.Thesis, University of Khartoum, Sudan.

Kwak, H. S.; Choi, S. S.; Ahn, J. and Lee, S.W. (2002). Casein hydrolysate fractions act as emulsifiers in process cheese. J. Food Sci., 67(2):821-825.

Lahtinen, S. J.; Forssten, S.; Aakko, J.; Granlund, L.; Rautonen, N. and Salminen, S. (2012). Probiotic cheese containing Lactobacillus rhamnosus HN001 and Lactobacillus acidophilus NCFM® modifies subpopulations of fecal lactobacilli and Clostridium difficile in the elderly. Age (Dordr)., 34(1): 133-143.

Law,B. A and Tamime, A.Y.(2010). Technology of cheese making, 2nd ed. Wiley and Sons, Ltd. Publication,UK.

Law,B.A.(1999). Technology of cheese making. Sheffield Academic Press, Sheffield, UK.

Levitan, E. B. A.; Wolk, andMittleman. M. A. (2009). Consistency with the DASH diet and incidence of heart failure. Arch. Intern. Med., 169:851–857.

Lollo, P. C. B.; Cruz, A. G.; Morato, P. N.; Moura, C. S.; Carvalho-Silva, L.; Oliveira, C. A. F.; Faria, J. A. F.;and Amaya-Fartan, J. (2012). Probiotic cheese attenuates exercise-induced immune suppression in Wistar rats. Journal of Dairy Science., 5(7):3549-3558. http://dx.doi.org/10.3168/jds. 2011-5124[Links].

Madureira, A. R.; Brandao, A. M.; Pintado, M. E.; Gomes, A. M. andMalcata, F. X. (2011). Technological optimization of manufacture of probiotic whey cheese matrices. J. Food Sci., 76:203-211.

Madureira, A. R.; Pintado, A. I.; Gomes A. M.; Pintado, M. E., and Malcata, F. X. (2011). Protective effect of whey cheese matrix on probiotic strains exposed to simulated gastrointestinal conditions. Lebenson. Wiss. Technol., 44:75-81.

Magdalena, A.; Catherine, S.; Lorenzo, M.; Gregor, R. and Maya, P. (2006). Probiotics in food health and nutritional properties and guidelines for evaluation, Combined Report of ajoint FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food including powder Milk with live lactic Acid Bacteria, Cordoba,Argentina.,45 (7): 110-124 .(2006).

Mahindru,S.(2009). Milk and milk products. Emertus Consultant Food Standards and laws MDH ltd.,A.P. H. Publishing Corporation. New Delhi, India.

McBean,L.D.(2002). Health benefits of cheese. National Dairy Council Dairy Council Digest., 73(5) :20-35.

McSweeney, P. L. H. (2011). In: Fuquay JW (ed) Encyclopedia of dairy sciences, 2nd edn. San Diego, Academic Press Medici, M., C. G. Vinderola, and G. Perdigon,(2004). Gut mucosal immune modulation by probiotic fresh cheese. Int. Dairy J., 14:611- 618.

Miller, G.D.;Jarvis, J.K. and McBean, L.D.(2000). Hand book of dairy food and nutrition.

Minervini, F.; Siragusa, S.; Faccia, M.; Dal Bello, F.; Gobbetti, M.; and De Angelis, M. (2012). Manufacture of Fior di Latte cheese by incorporation of probiotic lactobacilli. Journal of Dairy Science., 95(2): 508-520. http://dx.doi.org/10.3168/jds.2011-4150 [Links]

Mirzaei, H.and Barzgari, A. (2012). Isolation and molecular study of potentially probiotic Lactobacilli in traditional white cheese of Tabriz in Iran. Ann. Biol.Res., 3 (4): 2019–2022.

Modzelewska, M.; Kobukowski, J. and Kłebukowska, L. (2010). The influence of feeding diets containing white cheese, produced with prebiotics and the potentially probiotic *Lactobacillusplantarum* strain, on the gastrointestinal microflora of rats. Czech J. Food Sci., 28:139-145.

Nour-El,Diam, M.S.A and El Zubeir, I.E.M.(2007). Yield and sensory evaluation of processed cheese from Sudanese white cheese. Res.J of Animal and Vet Sci., 2 (1): 47-52.

Nuser, S.M.(2001). The effect of cooking and vacuum packaging on the quality of white soft cheese. M.Sc. thesis, University of Khartoum, Sudan.

Obando, C. M.; Brito, C. M.; Schobitz, T. R.; Baez, M. L. andHorzella,R. M. (2010). Viability of the probiotic microorganisms *Lactobacillus casei* 01, *Lactobacillus acidophilus* La-5, *Bifidobacterium* BB12 during cottage cheese shelf life. Vita., 17:141–148.

O'Connor,C.B.(1993). Traditional cheese making manual. International Livestock Centre for Africa. Addis Ababa, Ethiopia.

Oner, Z.; Karahan, A. and Aloglu, H. (2006). Changes in the microbiological and chemical characteristics of an artisanal turkish white cheese during ripening. LWT - Food Science and Technology, 39(5): 449-454. http://dx.doi.org/10.1016/j.lwt.(2006).03.015 [Links].

Ong, L.; Henriksson, A. and, Shah, N. (2007). Proteolytic pattern and organic acid profiles of probiotic Cheddar cheese as influenced by probiotic strains of Lactobacillus acidophilus, Lb. paracasei, Lb. casei or Bifidobacterium sp. Int Dairy J., 17:6778- 6790.

Ortakci, F.; Broadbent, J. R.; Mcmanus, W. R.; and Mchahon, D. J. (2012). Survival of microencapsulated probiotics Lactobacillus paracasei LBC-1, during manufacture of Mozzarella cheese and simulated gastric digestion. Journal of Dairy Science., 95(11); 6274-6281. PMid:22981567. http://dx.doi.org/10.3168/jds. [Links].

Osman, E.H.E.(2005). The effect of packaging on the quality of Sudanese white cheese. M.Sc.Thesis, Sudan University of Science and Technology, Sudan.

Ozer, B., and Kirmaci,H.(2009). Development of proteolysis in white-brined cheese: Role of microencapsulated *Lactobacillus acidophilus* LA-5 and *Bifidobacterium bifidum* BB-12 used as adjunct cultures. Milchwissens chaft., 64:295-299.

Papaioannou, G. I.; Chouliara, E.A.; Karatapanis, G.M.; Kontominas and Savvaidis,N.I.(2007). Shelf-life of a Greek whey cheese under modified atmosphere packaging. Int. Dairy J., 17: 358-364.

Parente E., and Cogan T.M. (2004). Starter cultures: General aspects. In: General Chemistry,Physics and Microbiology. Vol I, edited by P.F. Fox., P.J. H. McSweeney, T. M. Coganand T. P. Guninee. Amsterdam Elsevier.

Patricia,C.P.(1984). Making cheeses, butters, cream and yoghurt at home. Thorsons Publisher Lmited Welling borough, Northampton Shire. Fourth Impression. Great Britain.

Pauline, E. and Karin, R. (2006). Preparation of dairy products. Agrobook 36. Printed by: Digigrafi, Wageningen and the Netherlands.

Payane, A.J.W. (1990). Introduction to Animal husbandry in the tropics and subtropics. 4th edition. Longman, Singapore., 12:758-763.

Pinto, S.; Rathour,A.K.; Prajapati,J.P.;Jana,A.H and Solanky,M.J.(2007). Utilization of whey protein concentrates in processed cheese spread. Natural Product Radiance., 6(5):398-401.

Quinn,P.J.;Carter, M.E.; Markey, B. and Carter, G.R.(2002). Entero bacteriaceae. In: Clinical veterinary microbiology, Wolf Pub. Spain., 32 (7): 209-236.

Raff, M. T.; Tholstrup, S.; Basu, P.; Nonboe, M.; Tang Sørensen,P.; and. Straarup. E. M.(2008) A new probiotics cheese with antioxidative and antimicrobial activity. J. Dairy Sci., 87:2017-2023.

Ramakant,S.(2006). Production processing and quality of milk and milk products. International Book Distributing Co. Publishing Division.

Raynal, K.; Lagriffoul, G.; Paccard, P.; Guillet, I. and Chilliard, Y. (2008). Composition of goat and sheep milk products: an update. Small Rumin Res., 79:57-72.

Reid, G. (2005). The importance of guidelines in the development and application of probiotics. Curr. Pharm. Des., 11:11-16.

Richard, W. (1978). The cheese book. Published by: Chart well Book. India.

Rijkers, G.T.; S. Bengmark, P.; Enck, D.; Haller, U.; Herz, M. Kalliomaki, S.; Kudo, I.; Lenoir-Wijnkoop, A.; Mercenier, E.; Myllyluoma, S. Rabot, J.; Rafter, H.; Szajewska, B.; Watzl, J. Wells, D.; Wolvers, and J.-M. Antoine. (2010).Probiotics and immunosenescence: Cheese as a carrier. FEMS Immunol. Med. Microbiol., 59:53-59.

Rodrigues, D., T.; Rocha-Santos, A. P.; Pereira, C. I.; Gomes, A. M.; Malcata, F. X. and Freitas, A. C. (2011). Potential effect of FOS and inulin upon probiotics bacterium performance in curdled milk matrices. Lebenson. Wiss. Technol., 44:100–108.

Robertson, G. L. (2006). Food packaging: Principles and practices.J. Boca Raton., 42 (7):507-549.

Rose, M.; Haider, H.; Weiller,C. and Buchel, C. (2002). The role of medial temporal lobe structures in implicit learning. An event-related FMRI study. Neuron., 36:1221-1231.

Santosa, S.; Farnworth, E.and Jones, P. J. H. (2006). Probiotics and theirpotential health claims. Nutrition Reviews.,64(6):265-274.

Saxelin, M.; Korpela, R.; and Mäyrä-Mäkinen, A. (2003). In: Smith G (ed) Dairy processing. Improving quality. CRC Press, Washington.

Scott, R.(1986). Cheese making practice. 2nd edition. Elsevier Applied Science Publisher -London and New York.

Shah, N. P. (2007). Functional cultures and health benefits. International Dairy Journal.,17(11):1262-1277.

Shirashoji, N.; Jaeggi, J.J and Lucey,J.A. (2006). Effect of trisodium citrate concentration and cooking time on the physicochemical properties of pasteurized process cheese. J. Dairy Sci., 89:15-28.

Shukla,A.N.(2010). Textbook of dairy chemistry first publication. Published by Discovery Publishing House. New Delhi, India

Slashinski, M. J.; Curdy, M. C.; Achenbaum, S. A.; Whitney, L. S.; Guire, S. N.; Curdy, M. C.; Achenbaum, A. L.; Whitney, M. C. and Guire, M. C .(2012). Snake-oil, quack medicine and industrially cultured organisms. Biovalue and the commercialization of human micro biome research BMC Medical Ethics., 53:28-42

Sousa, M. J.; Ardo,Y. and McSweeney, P. L. H. (2001). Advances in the study of proteolysis during cheese ripening. Int. Dairy J., 11:327-345.

SPSS.(2014). Statistical Package for Social Studies Software (SPSS), data filescould contain multiple record types -version 16.0 and later run underwindows, Mac and Linux.

Subhasish,B. and Subhash,K.B.(2006).Milk and milk products. First ed. Published by Jaypee Brothers Medical Publisher. New Delhi, India.

Sulieman, A.E.;Mohamed, H.; Elterefi,M and Reem,E.K.(2005). Chemicaland microbiological characteristics of Maddafara cheese. SudanJournal of Agricultural Research 5: 73-81.

Suresh, K. S.; Krishnappa, S. and Bhardwaj, P. (2013). Safety concerns of probiotic use: A review. IOSR Journal of Dental and Medical Sciences., 12(1): 56-60.

Sveje, M. (2007). Probiotic and prebiotics eimproving consumerhealth through food consumption. Nutracoss,sept/oct (2007).,72 (8) :28-31.

International Commission of Microbiological Specification for Foods.(1980). Milk and milk products. In Microbial Ecology Foods., 11: 499512. Edit by Silliker Alas. Acad. Press. the Quality of White Soft Cheese. M.Sc. Thesis, University.

Talwalkar, A.; Miller, C. W.; Kailasapathy, K. and Nguyen, M. H. (2004).Effect of packaging materials and dissolved oxygen on the survivalof probiotic bacteria in yoghurt. International Journal of Food Science and Technology.,39(6): 605-611.

Thomas, Q. (2008). Dairy farm management. Delma Publisher. Albany, New York.

Toledo, E. M.; DelgadoRodríguez, R.; Estruch, J.; SalasSalvadó, D.; Corella, E.; Gomez-Gracia, M.; Fiol, R. M.; Lamuela-Raventós, H.; Schröder,

F.; Arós, E.; Ros, V.; Ruíz-Gutiérrez, J.; Lapetra, M.; Conde-Herrera, G.; Sáez, E.; Vinyoles, R.; and Martínez-González, M. A. (2009). Low-fat dairy products and blood pressure: Followup of 2290 older persons at high cardiovascular risk participating in the PREDIMED study. Br. J. Nutr., 101:59-67.

Tolle,A.C.(1980).The micro flora for the udder infectors including the bacteriological quality of the raw milk. International Dairy Federation Bulletin.

Vankerckhoven, V. G.; Huys, M.; Vancanneyt, C.; Vael, I.; Klare, M. B.; Romond, J. M.; Entenza, P.; Moreillon, R. D.; Wind, J.; Knol, E. Wiertz, R.and Pot. B.(2008). Biosafety assessment of probiotics used for human consumption: Recommendations from the EU-PROSAFE project. Trends Food Sci. Technol., 19:102–114.

Vesterlund, S.; Vankerckhoven, M.; Saxelin, H.; Goossens, S.; Salminen, and A. C. Ouwehand,(2007). Safety assessment of Lactobacillusstrains: Presence of putative risk factors in faecal, blood and probiotic isolates. Int. J. Food Microbiol., 116:325–331.

Walstra, P.; Jan, T.; Wouters,M and Geurts,T. J. (2006). Dairy science and technology. Boca Raton: Taylor and Francis Group, CRC Press.

Walstra, P.; Geurts, T.J.; Noomen, A.; Jellems, A. and Nan Boekel, M.A.J.S. (1999). Dairy technology principles of milk properties and processes. Marcel Dekker, Inc. New York.

Wang, H. K.; Dong, G. C.; Chen, Y. F.; Cui, L. M. and Zhang, H. P. (2010). A new probiotic Cheddar cheese with high ACE-inhibitory activity and γ-aminobutyric acid content produced with koumiss-derived *Lactobacillus casei* Zhang. Food Technol. Biotechnol., 48:62–70.

Warsama, L. M.; Elzubeir, I. E. M. and El-Owni, O. A. O. (2006). Composition and hygienic quality of Sudanese white cheese in Khartoum North markets (Sudan). International Journal of Dairy Science., 1: 36-43.

Wedajo, B. (2015). Lactic acid bacteria: benefits, selection criteria and probiotic potential in fermented food. Journal of Probiotics and Health., 3(2): 1-9.

Appendix No. 1

Sensory evaluation sheet for white soft cheese
Name……
Date ……

Samples	Color	Taste	Flavor	Texture	Saltiness	Overall acceptability
1.						
2.						
3.						
4.						
5.						
6.						
7.						
8.						
9.						
10.						

Key:

N	Color		Taste		Flavor		Texture		saltiness		overall	
1	Veryacceptable	9	Very palatable	9	Extremely intense	9	Very soft	9	Over salted	9	Highly palatable	9
2	Acceptable	7	Palatable Intense	7	Soft intense	7	Very palatable	7	Salted	7	Very palatable	7
3	Moderately acceptable	5	Moderately palatable	5	Moderately intense	5	Slightly soft	5	Moderately salted	5	Moderately	5
4	slightly acceptable	3	Slightly palatable	3	Slightly intense	3	Tough	3	Slightly salted	3	Slightly palatable	3
5	Not acceptable	1	Un palatable	1	Poor	1	Very tough	1	Un salted	1	Not palatable	1