CHAPTER ONE

Introduction

Livestock is the main agricultural resource in Sudan. Its population is 105 million heads distributed throughout the country according to climatic condition. However, milk production from these animals was estimated to 4391,000 tons (MIFR, 2015).

Food quality influence the value of a product for the consumer and comprises intrinsic product attributes like safety, sensory properties, convenience and health, and extrinsic attributes like how it is produced (Luning and Marcelis, 2007). Customer satisfaction is an important aim of all food producers. The important aspect contributing to this aim is consistent quality of the product, so that the expectations of the consumers are met every time they buy and consume the actual product variety (Kraggerud *et al* ., 2008) .

Packaging materials provide a means to preserve, protect, merchandise, market and distribute foods. They play a significant role in how these products reach the consumers in a safe and wholesome form without compromising quality (Raheem, 2012).

Cheese is a solid food made from the curdled milk of cows, goats, sheep or other mammals. The milk is curdled using some combination of rennet and acidification (Smith, 2005). There are several types of cheese, which are grouped or classified according to criteria such as length of ageing, texture, methods of making, fat content, animal milk, country of origin, etc. The method most commonly and traditionally used is based on moisture content, which is then further narrowed down by fat content and curing or ripening

methods (Rotaru *et al*., 2008). Cheese is highly concentrated product, which is rich in protein and minerals such as calcium and phosphorus, essential amino acids; therefore, it is an important food in the diet (Lakovechenko, and Arsenva, 2016). Cheese packaging is an integral part of processing operations and cheese preservation. Consumers more often directly purchase cheeses in the self-service section of the supermarket, along with other pre packaged fresh produces. It is thus necessary to package products in a way that makes it possible to preserve their quality (Floros *et al.*, 2000).

White Cheese has been traditionally manufactured in different areas in Sudan, Mozzarella and Romi cheeses have been introduced to Sudanese markets very recently, and research concerning them in Sudan is very limited.

1.1Research problems:-

In view of the continued research effort in the dairy milk products mainly cheese is packed in plastic and paper, therefore cheese has been involved in transmission of illness and food poisoning outbreaks. Packaging also affects quality of chemical and microbial composition of cheese.

1.2Research objectives:-

1. To study the chemical and microbiological composition of White Romi and Mozzarella, cheese under different packing materials.

2. To evaluate the effect of different packaging materials on the quality and safety these cheese.

CHAPTER TWO

Literature Review

2.1 Milk: -

Milk is an indispensable food item and is considered as nature's perfect food for human beings as well as other animals. Mammals secretes milk for the nourishment of their young ones and milk of animals like cattle, buffalo, goat, sheep, camel, yak, llama, etc. are being used as food for human beings (NZFSA, 2003).

2.2 Nutritional value of milk: -

Milk composition is affected by various factors, including stage of lactation, breed differences, number of calving (parity), seasonal variations, age and health of animal, feed and management effects including number of milking per day and herd size (Jenkins and McGuire, 2006).

2.2.1 Protein milk: -

Protein availability is defined as the amount of protein available to be absorbed and utilized in the human body, to the protein intake. Casein and whey proteins are the two major types available in milk in a ratio of 80% to 20%. Whey proteins (20% in milk) digest rapidly compared to casein proteins thus providing greater quantities of essential amino acids (Haug *et al.*, 2007). Cow's milk generally contains 30–35 g/L protein which is commonly divided into two classes on the basis of the solubility at pH 4.6, the insoluble caseins,

which represent 80% of total milk protein, and the soluble whey (or serum) proteins, which represent 20% of total milk protein (Tamime, 2009).

2.2.2 Fat milk: -

Milk fat is a concentrated form of energy and protects the body by insulating it against temperature and environmental changes. Milk fat is a carrier for fat soluble vitamins and essential fatty acids (Adolfson *et al.*, 2004). The bulk of the fat in milk exists in the form of small globules, called fat globules they have size ranging from 0.1 to 22 microns and dispersed as oil in water type emulsion. The surface of each fat globule is coated with an adsorbed layer of material, called fat globular membrane. This membrane consists a phospholipid-protein complex that stabilities fat emulsion by keeping the globules separately (Eckles and Macy, 2002).

2.2.3 Milk Lactose: -

Lactose is a milk sugar and is the carbohydrate nutrient in milk, lactose in milk has comparatively lower glycemic index compared to glucose or sucrose thereby making it suitable for diabetic people. It also helps in the absorption of calcium and magnesium and is less carcinogenic compared to other sugars. Lactose prevents infection by stimulating bifidobacterium in the colon thus improving colon health (Adolfson *et al.*, 2004). Active cultures in yogurt help digest lactose thereby making it suitable for lactose intolerant people (Saxelin *et al.*, 2003). Chemically lactose is composed of one molecule each of glucose and galactose, souring of milk is due to the production of lactic acid from lactose by lactose fermenting bacteria and it is important in the preparation of fermented milk products (Kutty, 2004).

2.2.4 Minerals and Vitamins of Milk: -

Milk contains a number of minerals; however, the total concentration is less than1%. Mineral salts occur in solution in milk serum or in casein compounds. The most important salts are those of calcium, sodium, potassium and magnesium (Saxelin *et al.,* 2003). Milk provides human the Vitamin B, is concerned with nervous control. The human need of this vitamin is thought to increase with the intake of sugar and other carbohydrates; there is some evidence also that it plays apart in protein digestion and metabolism. Cow milk is very high in riboflavin (Vitamin B_2), which affects growth. Vitamin C and D are not present sufficiently in either cow's milk or goat's milk, and any child that is bottle-fed will need supplements (Hennessy *et al.,* 2007).

2.2.5 Milk Enzymes: -

Indigenous milk enzymes are found in, or associated with various, casein micelles, milk fat globule membrane, milk serum or somatic cells and may originate from blood, somatic cells, the milk fat globule membrane (MFGM) or the cell cytoplasm. These milk enzymes can be used as indices of animal health or thermal history the milk, they can result in quality deterioration or induce desirable changes in milk and dairy products or they may also offer protective effects. Important indigenous milk enzymes, e.g. plasmin, lipoprotein lipase, alkaline phosphatase and Lactoperoxidase (Tamime, 2009).

2.3 Importance of milk: -

Fluid milk is not only nature's food for a new born infant, but also a source for a whole range of dairy products consumed by mankind. Fluid milk is about

87% water and 13% solids. The fat portion of the milk contains fat-soluble vitamins. The solids other than fat include proteins, carbohydrate, water-soluble vitamins and minerals. Milk products contain high quality proteins. The whey proteins constitute about 18% of the protein content of the milk. Casein, a protein found only in milk, contains all of the essential amino acids and accounts for 82% of the total proteins in milk. Milk also contains calcium, phosphorus, magnesium, and potassium. The calcium found in milk is readily absorbed by the body; Vitamin D plays a role in calcium absorption and utilization. Milk is also a significant source of riboflavin (vitamin B_2), which helps promote healthy skin and eyes also Dairy products such as yogurts, cheeses and ice creams contain nutrients such as proteins, vitamins and minerals. Consumption of dairy products been associated with decreased risk of osteoporosis, hypertension, colon cancer, obesity and insulin resistance syndrome (IRS) (Weaver, 2003).

2.4 Fermented milks:-

Fermented milk is a milk product obtained by fermentation of milk which milk may have been manufactured from products obtained from milk with or without compositional modification by the action of specific microorganisms and resulting in reduction of pH and coagulation. These specific microorganisms shall be viable, active and abundant in the product to the date of minimum durability if the product is not heat-treated after fermentation (CODEX, 2000).

IDF 1992 (International Dairy Federation), published general standards of identity for fermented milks that could be briefly defined as follows:

Fermented milks are prepared from milk and/or milk products (e.g. any one or combinations of whole, partially or fully skimmed, concentrated or powdered milk, butter milk powder, concentrated or powdered whey, milk protein (such as whey proteins, whey protein concentrates, soluble milk proteins, edible casein and caseinates), cream, butter or milk fat-all of which have been manufactured from raw materials that have been at least pasteurized) by the action of specific microorganisms, which results in a reduction of the pH and coagulation.

Ghana standard (2003) mentioned that many traditional fermented milk products were made in Asia, Africa, the Middle East, and northern and eastern Europe. Cheese is a solid food made from the curdled milk of cows, goats,sheep or other animals. The milk is curdled using some come combination of rennet (or rennet substitutes) and acidification. Bacteria acidify the milk and play a role in defining the texture and flavor of most cheeses. Adding acids such as vinegar or lemon juice can curdle the milk as well.

2.5 Cheese: -

De (1998) defined cheese is a product that made from the curd obtained from milk by coagulating the casein with the help of rennet or similar enzymes in the separation of milk, cream or partly skimmed milk, buttermilk or a mixture of these products , it can also be made from the milk of cows, sheep, goats and camels or mixture of two of these. Each type of milk imparts the characteristics quality of cheese made from it and the resulting cheese will diver in its proprieties, body texture, and flavor (Andrew, 2010).

The present word (cheese) is derived through the old English word Cese and Chiese from Latin Caseus, the equivalent word in German Kaise, in Spanish Quaso and in Italian Formaggio (Scott, 1986). Smith (2005) cheese is defined as the solid food made from the milk of cows, goats, sheep and other animals, and it is lighter weight, more compact and has a longer shelf life than the milk from which it is made. However, Alfa-Laval-Dairy Handbook, defined cheese as the fresh natural product obtained after coagulation of milk, cream, skim, or partly skimmed milk, buttermilk, or a combination of these products , cheese as the product made from the curd obtained from milk by coagulation of casein with the help of rennet or similar enzymes in the presence of lactic acid produced by added or adventitious microorganisms, from which part of the moisture has been removed by cutting, cooking and / or pressing, which has been shaped in a mould, and then ripened by holding it for some time at a suitable temperature and humidity.

2.5.1 History of cheese: -

Cheese is an ancient food there is no conclusive evidence indicating where cheese-making originated. The date of origin of cheese making ranges from around 8000 BC when sheep were first domesticated to around 3000 BC. The first cheese may have been made by people in the Middle East or by nomadic Turkish tribes. The earliest archaeological evidence of cheese making has been found in Egyptian tomb murals dating to about 2000 BC(Smith, 2005).

The earliest cheese was likely to have been quite sour and salty, similar in texture to rustic Cottage cheese or Feta (Smith, 2005). Factory made cheese overtook traditional cheese-making during the World War II, and factories

have been the source of most cheese in America and Europe ever since (Harold, 2004).

2.5.2 Classification of cheese: -

There are more than 400 different kinds of cheeses that can be made, According to Rodert and Thomas (2004) cheese classification falls into one of four main groups;soft, semi-soft, hard and very hard. This classification is based on moisture content in the cheese, thus the body and texture of cheese range from soft unripened cheese (such as cottage with 80% moisture) to very hard grated shaker cheese (such as Parmesan and Romano cheese). The ripened cheese has 32%-34% moisture. However De (1998) reported that there are probably about twenty distinct classes, types and varieties of cheese in the world today, although they are given over a thousand different names. Cheese is classified according (De, 1998): Geographical consideration: country, valley institution, town or region where first produced or marketed , Type of milk (cow, sheep, goat or buffalo). Method of manufacture: temperature of cooking, degree of acidity and firmness of cutting etc…which affect firmness (hardness / softness) , Chemical analysis: water, calcium, sodium chloride, casein, lactose, fat content and acidity and Microbiological properties: bacteria-ripened and unripened etc. and General appearance: flavour, size, color and keeping quality ,Physical and archaeological properties: very hard (less than 25%, moisture), semi-hard (36-40%) moisture) and soft (more than 40% moisture).

2.5.3 Cheese in Sudan: -

Milk and milk products seem to be of little importance in Africa at large particularly in Sub-Saharan Africa. However, south of the Sahara (desert) and the eastern flank and down to south of the Sahara are the most important milk areas (Dirar, 1993). Cheese is produced in Sudan throughout the country especially in Eldueim, White Nile State; Elobied, North Kordofan State; Nyala, South Darfur State and other localities in the country (El Owni and Hamid, 2007). However, cheese making in Sudan is the major preservation method or surplus milk in rural areas especially during rainy season when plenty of milk is available (El Owni and Hamid, 2007; El Owni and Hamid 2008).

Sudanese traditional fermented foods represent the main source of nutrition for rural and urban communities. Dairy product participated in enhancement of the economy, finance and business of local societies (Salih *et al*., 2011).

The major type of cheese in Sudan Jibna Beida ,beside Mudaffara and recently Gouda and Mozzarella cheese are introduced (Ibrahim, 2008). The cheese of Sudan is a product traditionally made from raw milk to which salt (6-20%) has been added without the use of starter culture and is usually packed in plastic containers, a practice which leads to deterioration of quality of. This high salt (6-20%) may lead to direct effect on health and blood pressure (Lifton *et al*., 2001).) High salting preserve cheese from rapid deterioration before ripens (Taormina, 2010).

2.5.4 Cheese microorganisms: -

2.5.4.1 Cheese spoilage and pathogenic microorganisms: -

Food spoilage is metabolic process that causes foods to be Undesirable or unacceptable for human consumption due to changes in sensory characteristics. However, spoiled foods may be safe to eat, i.e. they may not cause illness because there are no pathogens or toxin present, but changes in texture, smell, taste or appearance cause them to be rejected. Some ecologists have suggested these noxious smells are produce by microbes to repulse large animals, thereby keeping the food resource for themselves (Burkepile De *et al.*, 2006).

Microorganisms play a significant role in the spoilage of dairy products as well as in the ripening of some cheese varieties (Irlinger and Mounier, 2009). The spoilage organisms in cheese and dairy products are coliforms, yeast and moulds, propionic acid bacteria, faecal *Sterptococci, Lactococcuslactic* and *psychrotrophic* bacteria (Ceylan *et al.*, 2003). The type of spoilage microorganisms differs widely among dairy foods because of the selective effects of practices followed in production, formulation, processing, packaging, storage, distribution, and handling (Ledenbach and Marshall, 2009).

Many pathogenic organisms may be present in raw milk. They may be derived from an infected (mastitic) udder, the faeces or other excreta of infected cows or symptomless (carrier), cows, human sources, a contaminated environment or dairy equipment. The group includes *Staph, aureus, strepotococcus spp, salmonella,E. Coli, Listeria monocytogenes*, etc. If pathogens are present in such and survive the cheese making process, this may result in incidents of

food poisoning due to the ingestion of cheese contaminated with pathogenic organisms or with their entertoxins.Mould species are essential for ripening of specific varieties of cheese, but mould growth on most cheeses is undesirable. It spoils the appearance, can impart musty flavour, and may produce microtoxins (Siew *et al.*, 2004).

Unwanted gas production can occur during the manufacture or ripening of cheese depending on the number and bind of gas producing microorganisms in the milk, or contaminating the curd. Raw milk heavily contaminated with coliform bacteria may form so much gas that the curd floats in the vat. *Coliform bacteria* are able to tolerate the acid and salt conditions of most cheese ,cooking process helps to destroy spoilage microorganisms and improves the shelf life of the processed cheese. Cooking curd contracts the particles and drives out the free whey, influences texture, gives more time for production of lactic acid and also suppresses the growth of spoilage organisms in acid cheese (Nuser, 2001) .

2.5.4.1.1 *Escherichia coli: -*

Escherichia coli (*E. coli*), a member of the *Enterobacteriaceae*, are motile, non-spore-forming bacilli that stain Gram-negative. The bacterium lives commensally in the intestines of humans and warm blooded animals. *Enterohaemorhagic E. coli* (*EHEC*), which was first recognized as a cause of illness in 1982, has been held responsible for some cases of very severe poisoning that ended in death. *EHEC* includes *E. coli* strains (*E. coli* O157:H7) that produce verotoxins. Verotoxins have a cytotoxic effect and also produced by some other *E. colisero* types. Verotoxin-producing *E. coli* are considered a major cause of gastrointestinal disease in developed countries. The nature of illness can range from a mild form of diarrhea to more severe forms known as

hemorrhagic colitis and hemolytic uremic syndrome (Reed, 1994, Riley *et al*., 1983). Although the number of organisms required to cause disease is not known, it is suspected to be very small. The sources of infection are consumption of sprouts, unpasteurized milk and insufficient cooking or raw consumption of contaminated products increases the risk of poisoning. (Waites and Arbuthnott, 1991, Reed, 1994).

2.5.4.1.2 Bacillus species:-

Bacillus cereus is a group of ubiquitous facultative anaerobic spore forming, Gram-positive rods commonly found in soil, the spores frequently contaminate a variety of foods, like dairy products. Their ubiquity and the resistance of their endospores to the various operations used in food processing contribute to their presence in foods, while their ability to adapt to a wide range of temperatures, pH and nutrient sources promotes their multiplication. Several Bacillus species, mainly *Bacillus cereus*, have been implicated in foodborne gastroenteritis. The control of Bacillus spp. requires the inactivation of spores or the inhibition of cell growth using a variety of techniques (Jay *et al.,* 2005).

Bacillus cereus pores are common in food, as well as in the gastro- intestinal tracts of invertebrates and vertebrates including mammals, and bacterial spores may contaminate the food production chain from all of these sources (Jensen, *et al.,* 2003; Earl, *et al.* and 2008; Swiecicka, 2008).

In a dairy farm, spore concentrations in milk were correlated to spore concentrations in cow faeces, which were also correlated to the concentration of B. *cereus* in the cows' feed (Guinebretiere and Nguyen, 2003).

2.5.4.1.3 *Salmonella*:-

Salmonellae are medium size Gram- negative non spore forming rods. They are facultatively anaerobic with an optimum growth temperature of 37^{o}C. *Salmonellae* can survive for several months in micronutrients such as faecal particles, moist soil and stream sediments, has been a continued increase in milk production and consumption of fermented dairy products, Cheese is one of the most consumed milk. Family of *Enterobacteriaceae* contains many species which cause hazard to the consumer, other species are important from economic point of view as milk products. *Salmonellosis* may cause an outbreak of gastroenteritis (El-Kouly, *et al*., 1995). Cheese like other types of food is exposed to contamination with microorganisms during the production process. (Ahmed *et al*., 2000).

Since dairy products manufacturing, handling and distribution in Iraq are carried out under primitive conditions therefore *Salmonella* contamination was expected and, so it's highly recommended that strict hygienic conditions should be adopted during manufacturing and handling of such products, besides that local markets and processing should be periodically inspected by specialists (Ali, 2005).

2.5.4.1.4 *Staphylococcus aureus:-*

Staphylococcus aureus is non sporeforming; salt tolerant and facultative anaerobic Gram- positive coccus. The food handlers were found to be the source of contamination. Low numbers of *Staph. aureus* is relatively common in raw milk. This may due to contamination from the udder surface during milking, and/or shedding of the organism into milk by cattle with subclinical *staphylococcal mastitis Staph. Aureus* is able to tolerate salt and moderate

acidity, and can multiply during cheese manufacture and ripening in soft cheeses. It may survive for long periods even in hard cheeses, and, if high enough populations are developed, enterotoxin may be produced, which persists for many months even after the cells have lost viability (Simeao *et al*, 2002).

Staphylococcus aureus is a leading cause of gastroenteritis resulting from the consumption of contaminated food, contamination of dairy products with *Staphylococcus aureus* bacteria may influence considerably their harmlessness, decrease their shelf quality and endanger the health of consumers of *Staphylococcus aureus* causes disease both in people and animals (Moretti *et al.*, 1998, and Nishijima *et al.*, 1997). *S. aurous* can gain access to milk either by direct excretion from udders with clinical or subclinical staphylococcal mastitis or by contamination from the environment during handling and processing of raw milk (Peles *et al.*, 2007).

2.5.4.1.5 *Listeria monocytogenes*:-

Listeria monocytogenes Gram-positive, non-sporeforming rods, the genus Listeria consists of at least7species of. Only one species, *L.monocytogenes*, is considered a human pathogen. In human adults, *L. monocytogenes* is known to cause meningitis, encephalitis, abscesses, and death (Kiiyukia, 2003). It is widely spread in nature, easily enters the food and as such can lead to contamination of the food. The bacteria were isolated in milk and dairy products. Raw milk is one of the most common paths for *transmissionL. monocytogenes*, mainly due to sick animals on the farm. It is important to point out that healthy animals are often carriers of *L. monocytogenes* and as such can be source of contamination of the environment, or milk and cheese. (McLauchlin *et al.*, 2004). *Listeria* are associated with dairy products, where

cow milk is mentioned as carrier of the fatal listeriosis. Of all dairy products, cheeses and non-pasteurized milk are most common causes of listeriosis. Two large outbreaks in human population were associated with consumption of cheeses (Farber and Peterkin, 1991).

2.5.4.1.6 Coliforms:-

Coliforms bacteria are main contaminants of raw milk and dairy Products, including fresh cheeses. The *coliforms* are easily destroyed by heat treatments usually employed for milk, being an indicator of process failures or post-processing contamination in pasteurized foods. *E. coli* is considered as a harmless bacterium that are most often used as indicator organisms for fecal contamination and breaches in hygiene. However, several *E. coli* clones have acquired virulence factors that have allowed them to adapt to new niches and in some cases to cause serious disease (Okura *et al*., 2010).

In cheese production, slow lactic acid production by starter cultures favors the growth and production of gas by *coliform* bacteria, with coliforms having short generation times under such conditions. In soft, mold-ripened cheeses, the pH increases during ripening, this increases the growth potential of coliform bacteria (Frank, 2001). Contamination of cheese with *coliforms* especially fecal coliforms gives an indication of either direct or indirect fecal contamination and considered as a mirror for the degree of disregard of numerous hygienic rules during processing and marketing (Jay, 2000).

2.5.4.1.7 Lactic Acid Bacteria:-

Lactic acid bacteria (LAB) are ubiquitous microorganisms that can be beneficial in crop and livestock production, with their long history of use in

food preservation by many world cultures. LAB are generally recognized as safe for human consumption, by producing lactic acid as a fermentation metabolite, these microorganisms prolong storage, preserve nutritive value, and enhance flavors of otherwise perishable foods (Nordqvist, 2004).

Heterofermentative lactic acid bacteria such as *lactobacilli* and *Leuconostoc* can develop off flavors and gas in ripened cheeses. These microbes metabolize lactose, subsequently producing lactate, acetate, ethanol, and CO2 in approximately equimolar concentrations (Hutkins, 2001). Their growth is favored over that of *homofermentative* starter culture bacteria when ripening occurs at 15°C rather than 8°C (Cromie *et al.*, 1987). When the *homofermentative lactic acid* bacteria fail to metabolize all of the fermentable sugar in a cheese, the *heterofermentative* bacteria that are often present complete the fermentation, producing gas and off flavors, provided their populations are 106 CFU/g (Johnson, 2001).

2.5.4.1.8 Yeasts and moulds: -

Yeasts are found in a wide variety of cheeses, however, in most cases, their role in cheese ripening is unclear. The low pH, low moisture content, low temperature and high salt level of ripening cheese favor the growth of yeasts. *Yeasts* are not usually used in milk processing and are normally regarded as spoilage organisms in dairy products Moreover, some yeast species capable of causing bitter taste, putrefaction and gas formation in Turkish white cheese were identified (Roostita and Fleet, 1998). However, spoilage of dairy products due to excessive growth of yeasts has been reported and they can cause fruity flavor, gas production, discoloration, slime formation and changes in texture (Westall and Filtenborg, 1998; Jacques and Casaregola, 2008).

Kurtzman *et al.*, (2011) reported that *yeast* colonies were characterized phenotypically according to standard methods currently used in *yeast* taxonomy.

O'Connor (1994) reported that, moulds are aerobic organisms and their growth on foods can be reported by excluding air though careful packaging, they can be killed by relatively mild heat treatment, but mould spores are more resistant to heat. *Moulds* are important in the ripening of cheeses. *Moulds* ripened cheeses are divided into two groups: those which are ripened due to the presence of *pencillium roqueforti* which grows and forms blue veins within the cheese such as Roqueforti, Gorgonzola, Stilton and Danish blue, and the second group are those which are ripened with *Pencillium camemberti* which grows on the surface of the cheese such as Camembert and Brie (Gripon, 1993).

Moulds cause major changes in the food products during their storage, which makes them unsuitable for consumption due to reduction of their nutritional value or accumulation of mycotoxins. *Mould* growth on cheese is a common problem during maturation at dairy processing facilities, and also in retail products for the end user during prolonged refrigerator storage. The *moulds* of the *Penicillium* and the *Aspergillus* genera are the most common contaminants (Gandomi *et al.*, 2009).

Mould growth can then be observed on the cheese during ripening, during storage at the factory or during retail distribution. The cheeses become spoiled due to the visible *mould* colonies on the surface and the off-flavors they produce. The *mould* growth may also represent a health risk because of the

possibility of mycotoxin production by some *mould* species (Kure and Skaar, 2000).

2.6. Microbial quality of cheese: -

The microbial quality and safety cheese is the major area of concern for producers, public health authorities and consumers. It depends on the types of microorganisms introduced from raw milk, efficiency of processing and the hygienic practice applied in small or big dairy plant or informal producers. Handling of milks during cheese manufacture play an important role in the proliferation of microbial flora and consequently impair its utility and render the product unfit for human consumption (Aly and Galal., 2002).

The microbiological quality of the milk and the good manufacturing practices will contribute to the safety of the final product; especially in cheeses where milk is not pasteurized (FDA, 2012). Most cheese is now produced from milk that has been pasteurized and it is indisputable that some outbreaks of food-borne illness have been clearly linked with the consumption of cheese, the majority of those reported being associated with cheese made from unpasteurized milk, Whilst pathogens can gain access to cheese after curd formation, moreover, they showed different types of the potentially foodborne disease (*E. coli*, *Salmonella spp.* and *S. aureus*) which were found in cheese, unless milk used for cheese processing is pasteurized or otherwise treated to destroy pathogens, pathogenic or toxin producing organisms present in raw milk could be found in cheese (Jay,2000).

Ceyhan *et al*, (2003) studied the dairy industry has had some unfortunate experiences with respect to the presence of pathogenic bacteria in cheese.

Pathogens such as *Listeria monocytogenes*, *Yersinia enterocolitica* and *Escherichia coli* O157:H7 have been involved, along with the more familiar *Staphylococcus aureus*. The dairy industry has responded by further upgrading its already high standards; it has improved processing procedures, sanitation, established good manufacture practices and implemented Hazard Analysis Critical Control Points (HACCP). Fungal growth on cheese is a common problem for the cheese manufacture during ripening and curing as well as for the retailer and consumer during refrigeration storage. Species of *Penicillium* and *Aspergillus* are the most common contaminants of cheese (Gandomi, *et al*., 2004). By the searching in the medical references, it was observed that, most of these fungi had the ability to human and animal pathogenicity or produced toxins (Ghibaudo and Peano, 2010).

2.7 Sensory characteristics of cheese: -

Sensory characteristics of food play an essential role in consumer behavior, particularly choice and the decision to purchase in the market place. Sensory attributes (flavor and texture) are critical to identify cheese and consumer acceptance, although, many approaches have been developed to evaluate the sensory attributes of foods (Drake, 2004). The flavor and texture are considered as the two main criteria in determining the acceptability of aged cheese. The time that is required to develop characteristic flavor and texture varies from a few weeks for soft cheeses up to three years for very hard varieties. During this period, cheeses attain their own characteristics through a multitude of chemical, microbiological and biochemical changes where- by protein, fat and residual lactose are broken down to primary products which are further degraded to secondary products (Engels *et al*., 1997, Amos, 2007).

Microorganisms are an essential component of all natural cheese varieties and play important roles during both cheese manufacture and ripening. Cheese is a very involved microbial ecosystem, and a very complex micro flora develops in most cheese varieties. Microorganisms, present in cheese throughout ripening, play a significant role in the ripening process, and selection of suitable strains would enable the cheese maker to control or modify flavor development. However, due to the complexity of the flora and the interactions which occur be- tween individual components of it and the cheese environment, strain selection for flavor improvement is not always very obvious. The advent of molecular techniques to study cheese micro flora will lead to a major in- crease in our understanding of this ecosystem and this knowledge will be harnessed to control cheese ripening (Amos, 2007, Beresford *et al*., 2001).

During the ripening of cheese, three major biochemical events occur (glycolysis, lipolysis and proteolysis), each of which is involved in flavor formation. The latter is the most important and also the most complex. Glycolysis is the conversion of lactose to lactic acid and is due to the growth of starter bacteria, and the lactate produced gives the freshly made cheese its overall acidic taste. They can also produce diacetyl, acetate and ace- taldehyde, which are important, compounds in flavor formation in fresh cheeses; diacetyl is also an important flavor compound in hard cheeses. Lipolysis results in hydrolysis of the milk fat and production of glycerol and free fatty acids, many of which, particularly the short-chain ones, have strong characteristic flavor. The fatty acids can be further metabolized to methyl ketones and fat also acts as a solvent for many of the flavor compounds produced in the cheese (Engels *et al*., 1997, Cogan and Beresford, 2002, Mcsweeney, 2004).

2.8 Packaging: -

Packaging or packing is one of the more important steps in the long journey from the producer to the consumer, since most of the cheese plants are far away from the consumption. The package may also serve as a processing aid for instance the metal can be used in heat sterilization of many food items. Also may prevent moisture loss, improve appearance, protect against microorganisms, and prevent oxygen transmission also may serve as a marketing tool, which provide useful information about the producer name, brand size, variety, net weight, count, shipper and country of origin. Also the nutrition information, recipes, and shelf life also become an important part of point of sale displays (Sacharow and Grffin, 1980).

The food industry has seen great advances in the packaging sector since its inception in the 18th century with most active and intelligent innovations occurring during the past century. These advances have led to improved food quality and safety. While some innovations have stemmed from unexpected sources, most have been driven by changing consumer preferences. The new advances have mostly focused on delaying oxidation and controlling moisture migration, microbial growth, respiration rates, and volatile flavors and aromas. This focus parallels that of food packaging distribution, which has driven change in the key areas of sustainable packaging, use of the packaging value chain relationships for competitive advantage, and the evolving role of food service packaging (Brody *et al*., 2008).

2.8.1Functions of packing:-

Packing is the protection all kinds of food by vessels have been designed to isolate the content of external influences, a coordinated system of preparing goods for transport, distribution, storage, retailing and end use, means of ensuring safe delivery to the ultimate consumer in sound condition at optimum cost. The packaging has a very important role in keeping food products (naturals or manufactured), making it easier to transfer and circulation from the producer to consumer Where its positive impact on the nutritional, health and economic aspects, the main purpose of food packing is to protect the food from microbial and chemical contamination, oxygen, water, vapour and light **(**Eltoum *et al.*, 2013).

The packing prevent the food from mechanical damage caused during handling and transport by affect the permeability of water vapor, gases (e.g. O_2, N_2, CO_2, H_2) or volatiles in or out weight loss or uptake might be controlled, as is the shelf life by creating a low-oxygen atmosphere in modified atmosphere packaging provide an effective barrier against temperature, light or microorganisms; ensure chemical compatibility between packaging material and contents as no hazards must arise from a presumable leaching of toxic substances out of the packaging material and into the product affect the growth rate of microorganisms inside the packages by controlling the permeability of gases (Fellows, 2000; Brennan and Day, 2006). 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 food packaging not only targets convenience and protection properties but also presents many other applications (Han, 2005).

2.8.2Packaging materials:-

2.8.2.1Tin plate containers:-

Tin plate is light gauge, steel sheet or strip, coated on both sides with commercially pure tin and has been used for well over a hundred years as a robust form of food packaging. The use of tin plate for food and beverage packaging will result in some dissolution into the food content, particularly when plain uncoated internal surfaces are used. The recommended maximum permissible levels of tin in food are typically 250 μg/kg. The highest levels being found in products packaged in un-lacquered or partially lacquered tin plate cans (Wallace and Blunden, 2003).

Dissolution of the tin plate depends on the food matrix, acidity, presence of oxidizing reagent (nitrogen, iron and copper), and presence of air (oxygen) in the headspace, time and storage temperature. To reduce corrosion and dissolution of tin now a days cans are usually lacquered (Dvorzak and Perring, 2002). Metal tin containers whether lined or non-lined would secure safety measures for cheese during storage for maturity or for exportation purposes (Abdalla *et al.,* 2017).

2.8.2.2 Plastic containers:-

There are more than thirty different plastics in packaging; the most common are polyolefins, polyvinyls and polyesters. There are possibilities that chemical contaminants in plastic packaging intended for recycling may remain in the recycled material and could migrate into the food. Other aspects of plastics recycling, such as microbial contamination and structural integrity of the recycled plastic, are also important considerations for the safe use of recycled

plastics for food contact applications Plastic recyclers must be able to demonstrate that contaminant levels in the reformed plastic have been reduced to sufficiently low levels to ensure that the resulting packaging is of purity suitable for its intended use. The production of a polymer with the desired qualities will require additional antioxidants, processing aids, or other adjuvants. That may need to be added to the recycled polymer (CFSAN, 2006).

Plastics and polymers have become a part of our life today. In fact they have become as essential to mankind as food and water. There is no sphere of human activity in which plastics have not made their entry ranging from agriculture, chemical industry, and packaging, etc... (Idris and Alhassan , 2010). Generally, come across are not considered to be toxic or harmful in any way (Singh 2001). Plastics such as polyethylene (PE), polypropylene (PP), oriented polystyrene, Nylon, Polyester, Polyurethane, Saran and polyethylene terephthalate (PET) are used as food packaging material (Sacharow and Griffin, 1980).

2.8.2.3 Paper and glass containers:-

There is enormous variety of food packaging used ranging from, paper and paperboard (Eltoum *et al*., 2013). Paper pack (from stems of papyrus in ancient Egypt) is the oldest form of what is referred to as "flexible packaging". It was reported that sheets of treated mulberry bark were used as a flexible packaging material by the Chinese to wrap foods as early as the First or Second century during the next fifteen hundred years, the papermaking technique was refined and transported to the Middle East, then Europe and finally into the United Kingdom (Welt, 2005).

Paper and paperboard are sheet materials made from an interlaced network of cellulose fibers derived from wood by using sulfate and sulfite. The fibers are then pulped and/or bleached and treated with chemicals such as slimicides and strengthening agents to produce the paper product. Paper and paperboards are commonly used in corrugated boxes, milk cartons, folding cartons, bags and sacks, and wrapping paper. Paper and paperboards provides mechanical strength, they are biodegradable and have good printability. Coatings such as waxes or polymeric materials can be used to improve their poor barrier properties. Apart from their poor barrier properties to oxygen, carbondioxide and water vapour other drawbacks include their being opaque, porous and not heat sealable **(**Raheem, 2012). Paper products - Paper like webs of mixed cellulose and plastics, papers made from plastics, bonded fiber plastics, cloths and scrims, spun bonded fabrics, regenerated cellulose films, aluminum and steel foils (Marsh and Bugusu, 2007).

Glass container is made from base materials (limestone, soda, sand and silica), which were in plentiful supply, all ingredients were simply melted together and molded while hot. Although the mixing process and the ingredients have changed very little, the molding techniques have progressed dramatically (Welt, 2005).

2.8.3 Cheese Packaging: -

Packaging of cheese must afford general protection of the product from mechanical damage and poor environmental conditions during handling and distribution. Recently, the consumer desire for healthier and safer foods increased (Abdalla *et al* ., 2013).

The increase in cheese production in Sudan witnessed a retreat in packaging, as the metal containers were reused several times and sealed by soldering. However, soldering of cheese metal packages was prohibited and accordingly the packaging of cheese was changed to metal and plastic press lid containers (Idris and Alhassan, 2010).

2.9 Mozzarella cheese:-

Mozzarella cheese has become one of the most popular cheese varieties in the world because of its primary use in Pizza preparation. It usage is expected to grow as global interest due to the ever increasing demand for pizza and other foods that use Mozzarella. Buffalo milk is preferred for Mozzarella due to high fat, vitamin (A), protein and low cholesterol (Zicarelli, 2004). Hicsasmaz *et al.*, (2004) defined the functional quality of Mozzarella cheese by its ability to melt and stretch. The consumer preference to Mozzarella cheese depend on melting quality of the cheese therefore, the melt ability of the cheese is an important factor in determining the quality of cheese. Because the main application of mozzarella cheese is used as a topping on pizza, therefore the texture and particularly melting characteristics of cheese has significant effect on consumer acceptance. Mozzarella has a several desirable properties, such as medium firmness, appropriate melting and stretchability, and easy shredding (Jana and Mandal, 2011).

Differences in fat level and hence protein to fat ratio, that occur in milks have market influence on composition yield, archeology flavour and sensory attributes of cheese (Guinee, 2004). Legend has that Mozzarella firstly made accidentally in Naples South of Italy when cheese curds accidentally fell into a pair of hot water, also Mozzarella firstly made from the rich milk of water

buffaloes in Naples and now made from cow milk and milk powder, it differs from most cheeses in that it is usually consumed in the melted state (Kuo *et al*, 2011). Lambert, (2013) reported that fresh Mozzarella is generally white, but may vary seasonally to slightly yellow depending on the animal's diet and it is a semi-soft cheese. Low-moisture Mozzarella can be kept refrigerated for up to a month, though some shredded low-moisture Mozzarella is sold with a shelf life of up to six months.

2.9.1 Mozzarella cheese making: -

The cow and buffalo milk used for manufacturing mozzarella cheese were standardized at 3.5 and 6.0% fat content respectively with the skim milk of particular milk. Cow and buffalo milk were mixed in individual combination by standardizing cow and buffalo milk to 3.5 and 6.0 fat per cent, respectively and pasteurized in an open pan for 15 minutes on a gas burner at 61°C. Pasteurized milk was then, cooled to 31°C. The Lactic acid starter culture designed for Mozzarella cheese was added to the cheese milk of 0.04%, followed by addition of CaCl2 0.03%. After 15 min of ripening, Rennet was added with 0.019%. The curd was formed in 20-25 min and then, cut with wire knife horizontally and vertically in a cube form and allowed to heal for 15 min. It was then cooked at 45°C for 2 hours and developed acidity of whey with 0.18 to 0.19%. The curd was piled in the center of vat. The curd slab was turned every 15 min until the curd reached at desired pH 5.2 to 5.6. The whey was then, drained and the curd slabs were heated in tap water up to 70°C-80°C, stretched manually for 7 min and molded. The molded cheese was cooled in cold water at 4°C for 2 hour and brined in 23% brine solution for 2 hr. The cheese was packaged and stored at 4°C (Fasale *et al* ., 2017).)

2.9.2 Quality of Mozzarella cheese:-

.Ahmed (2000) reported that total solids of whole milk cheese was (49.3%). the acidity of cheese prepared from cow milk and buffalo milk was 0.8 and 0.7%. Islam (2006) found that the Mozzarella cheese of cow milk contained 21.9% protein, 5.5% lactose, pH 5.4%, 2.2% ash in cow milk Mozzarella cheese, 53.2% total solids in mozzarella cheese of cows' milk, reported fat content of Mozzarella cheese were (24.8%).

Fasale *et al* (2017) studied the yeast and mold count is presented initially in first 45 days the yeast mold count was not detected. After 60 days, the growth count ranged from 1 to 2.3 cfu/ml the yeast and mold count of less than 10cfu/ml in mozzarella cheese is acceptable Table Food Safety Standards (2011). After 15 days the TPC were detected in all the samples at 102 dilutions. After final microbial test of 60 days the highest TPC counts were found 28×103 respectively in sample having more proportion of cow milk which leads to high moisture content in mozzarella. So, due to the high moisture content the microbial growth was higher than other samples. In the other side least TPC counts were found 17×103 and 15×103 which had higher proportion of buffalo milk mozzarella having low moisture content than others which affects the growth rate of microbes. The maximum limit for TPC in mozzarella cheese was 50000 cfu/ml as reported in Food Safety Standards, (2011).

2.10 White cheese: -

White Cheese has been traditionally manufactured in which cheese is packed in tins, cans and re-used petroleum gallons, which are hermetically sealed any

type of sterilization, therefore cheese have been without emphasized in the transmission of illness and food poisoning outbreaks, is a rich source of minerals, protein, vitamin, fat and carbohydrate .In general, white cheese supplies a great deal of calcium and phosphorous Introduction Cheese is known to be of great nutritional value for human consumption as its fat and protein have a high biological value and contains all essential fatty and amino acids. Also it is a source of vitamins and minerals (Dhuol and Hamid, 2014).

The highest production of cheese is during the rainy season (El Owni and Hamid, 2007). They are varying in composition, texture, colour, taste and flavour. The variation is due to composition of milk, methods of production, microbial flora, type of package, microbial activity during ripening and ripening conditions. Cheese manufacturing is influenced by product composition, processing, packaging and storage conditions, control of temperature and humidity and transportation are dynamic aspect of health hazards (Nour El-Diam and El Zubeir, 2006).

2.10.1 White cheese making:-

Cheese was manufacture according to the method described by Ibrahim (2003) with some modifications. One hundred twenty liters (120 liters) of fresh clean cow's full cream milk was divided into four equal volumes (15 liters each) and kept into three separate tanks. The different milk sample was laboratory pasteurized at 72oc for 1 minute. The milk samples were then transferred into stainless steel containers for cheese manufacture and then cooled to 42 ºC. Commercial starter (*Streptococcus thermophillus and Lactobacillus bulgaricus*) in the ratio of 1:1% concentrate was added at the level of 1% (W/v). The milk was stirred gently for 15 minutes to avoid creaming before

renneting. Rennet powder (1 gram/50 liters) was dissolved in 50 ml of distilled water and added to milk at 40 oc. Fine Calcium chloride was added at the levels of 0.02 % immediately. Milk was then stirred for 20 minutes and then left undisturbed for 3 hours to develop curd. The curd was cut into small cubs (2.5x2.5x2 cm) .After draining, salt at 2% (w/v) was mixed with the curds .The curd was poured into small clean wooden molds lined with cheese cloth and press by (30 kg) weight overnight. The next day, brine solution was prepared by adding salt to the collected whey (8 % w/v), then pasteurized at 72°C for 1 minute and cooled to 40°C. The pressed cheese was cut into small cubes and then transferred to the triplicate sterile plastic buckets containers filled with whey. The Containers were sealed and stored at room temperature (38±2) for 90 days.

2.10.3Quality of White cheese:-

Warsma (2003) studied the chemical composition of 36 samples of white cheese in Khartoum North market, the result showed that total solids, total protein, fat, ash and acidity were found to be 47.8, 15.9, 14.0, 6.2 and 0.4, respectively. Hamid (2005) studied the microbiological properties of Sudanese white soft cheese and he stated that total bacterial count, *coliform* and *moulds* and yeast of cheese samples were increased significantly ($P \leq 0.05$) at the beginning of the storage then decreased towards the end of the storage period, *Staphylococcus aureus* was detected only at day zero and completely disappeared through the storage period. *Salmonella* was not found. Ceylan *et al*. (2003) studied the microbiological quality of White cheese and found that the average *coliform* count was 5.99 log cfu/g, the high coliform content was attributed to the post-contamination during storage. (Zottola and Smith, 1993)

said that gas forming by salt-tolerant lactobacilli over 10^3 colonies per milliliter of brine is considered dangerous. Total *yeast* counts must be less than 400 cfu/g and total mold counts must be less than 10 cfu/g (Cogan, 2011).

2.11 Romi cheese: -

Romi is fermented hard cheese manufactured from raw cow and buffalo's milk in Egypt. Environmental conditions prevailing during cheese ripening, combined with the composition of the cheese often create possibilities for extensive development of *mould* on cheeses surfaces, which reduces quality. As a result of *mould* growth mycotoxins may be produced in cheese, rendering it unfit for human consumption (Pitt and Hocking, 2009).

Roumy cheese, the main traditional hard cheese in Egypt, is manufactured in a high proportion under artisan conditions from raw cow's or mixture of cow's and buffalo's milk without using starter cultures and marketed when it has a queried sharp flavor closed to kefalotyic cheese after 3 to 6 months (Dabiza and Dabiza,2007, Hattem *et al* .,2012). Roumy cheese are placed in natural caves in the production area, where ripening takes place at a nearly constant relative humidity (90% - 95%) and temperature ($9^{\circ}C$ - $12^{\circ}C$). Under these conditions, *Penicillium roqueforti* enters the cheese and develops in the matrix, providing the final product with its characteristic appearance. Micro-biologically, this cheese offers a complex habitat in which prokaryotic and eukaryotic populations interact and develop throughout manufacturing and ripening (Florez and Mayo, 2006).

2.11.1 Romi Cheese making:-

The procedure suggested by (Abou-Donia, 2002), for making Ras cheese was adopted. Standardized milk (3.5% fat) was heated to 32ºC and sufficient rennet was added in the proportion of 2.5g per 100kg milk to complete coagulation in 30 – 40 minutes. The coagulum was cut into small pieces about the size of chickpea grains and then vigorously stirred. The temperature of the vat was then raised to 45ºC over a period of around 40 – 50 minutes, and gently stirring was maintained throughout. After the curd had settled, the whey drained out (acidity≈0.14%), salt was sprinkled over the curd at a ratio of 1% (w/w), and the curd was manually pushed to the sides of the fat. Molds, lined with cheesecloth, were filled with sufficient curd to produce one finished cheese, and manual pressure was applied to expel some of the remaining whey. Light mechanical pressure follows over the next four hours at which point the cheese was reversed in the press and left under pressure for overnight (approximately 18 hours). The cheese wheels were then removed from the moulds and cloths and placed in the salting chamber. After draining for a further day at ambient temperature, the surfaces of each cheese wheels were coated with a small amount of dry salt. By the following day, most of this salt will have been absorbed into the cheese, so that the wheels were turned and the dry salting process repeated once again. This dry salting produce was continued for a period of around forty-five day, either every other day or once every three days. After completely salting process, the surface of cheese wheels was painted by clove oil and ripened at 60°F (15.5°C).

2.11.2 Quality of Romi cheese:-

Dabiza and El-Deib, (2007) reported the chemical composition of Ras cheese, moisture was 47.46%, fat was 36.00% and protein was 26.78%, total nitrogen and soluble nitrogen were 16.01% and 7.31% , respectively). Abbas *et al*, (2017) reported the, changes in some chemical composition of Ras cheese PH , Moisture content, Fat , Protein and Salt, during the ripening period 30 days was 5.39± 0.1% , 34.51 ± 0.8 % , 34.78 ± 2.5% , 24.37± 0.9%, , 04.32± 0.2% respectively. the chemical composition of Ras cheese sample collected from markets the mean value of moisture content was 35.96%. The average of titratable acidity, of Fat, of Fat/dry matter, of salt, and of salt in water phase, were, 1.51%, 35.27%, 52.21%, 3.59% and 10.20%, respectively. (Osman *et al* .,2011).

Dabiza and El- Deib (2007) stated counts of different microbial groups in cheese during ripening are illustrated coliforms and total staphylococci disappeared after 30 days and molds disappeared after 15 days from the probiotic cheeses. The cheeses were free from *yeasts*. Ras cheese throughout ripening and storage periods. While, Shehata *et al*., (2004) found that yeast and *mould*, proteolytic, psychrophilic and viable spore forming bacterial counts significantly decreased along the ripening period of Ras cheese. They also found that Ras cheese containing bifid bacteria had the lowest viable undesirable bacterial counts of *yeast* and *moulds* counts during the ripening period.

Osman *et al*., (2011) studied the microbiological Characteristics of Ras Cheese Collected from Assiut Markets mentioned the microbiological analyses revealed that the average bacterial count in the cheese sample was

46.12 x 106 cfu/gm and 26.27 x 106 cfu/gm. A wide variation in the total bacterial count was found among the different cheeses samples. *coliform* bacteria were detected 50 cfu/gm in few samples, the numbers of Yeasts and Moulds ranged from 0.00 to 24.0 x 105 cfu/gm. Some of these microorganisms participate in the development of flavour, body texture, and some of them are undesirable which cause some defects in this cheese. Owing to uncontrolled hygienic conditions, it can be contaminated by *moulds* and *yeasts*. Therefore, the final flavor and texture of Ras cheese is influenced by the action of this flora. (Ayad *et al*., 2004). Also some fungal growth of the surface of roumy cheese looks like a white felt after a few weeks of storage such as *Geotrichumcandidum*, *Aspergillusochraceus*, *A. alliaceus*, *A. oryzae*, *A. niger*, *A. nidulans*, *Emericellanidulans*, *A. flavus*, *A. glaucus*, *Penicillium sp*., *Mucor sp*. and *Rhizopusstolonifer* (El-Fadaly *et al* .,2015).

Awad *et al*., (2012) reported, Factory air was identified as an important source of contamination of Ras cheese.(Kure *etal*.,2004) reported the In Roumy cheese, *Penicillium* species, including *P. commune*, *P. roqueforti* and *P.verrucosum* ,were identified as the dominant spoilage organisms, being isolated from over 70% of samples. Ochratoxins are a group of mycotoxins produced as secondary metabolites by several fungi of the *Aspergillus* species as *A.ochraceous* (Hayaloglu and Kirbag, 2007).

CHAPTER THREE

Materials and Methods

This study was carried out at Khartoum state to evaluation the effect of packing materials on the chemical composition and microbiology quality of White, Romi and Mozzarella cheese in different packing materials from different area Khartoum , Khartoum North and Omdurman. The manufacture date of the collected cheese samples was defined to be at fixed date.

3.1 Expermental Design:-

A total of 300 samples (120 samples; 20 samples from plastic pack and 20 samples from paper packg) was collected randomly from supermarkets in three different areas; Khartoum North, Omdurman and Khartoum from each of white, Romi and Mozzarella cheeses with different packing materials except for Mozzarella only one package was found, and carried in cold ice box to the laboratories for chemical and microbiological analysis, at the laboratory of Dairy Production Department, College of Animal Production at Khartoum University, during the period from November 2016 to December 2017.

3.2Analysis of cheeses:-

3.2.1 Chemical analysis:-

3.2.1.1 Titratable acidity:-

The titratable acidity (TA) of cheese was determined according to the AOAC (2009). Ten grams of minced cheese were weighed in to a conical flask, and distilled water at 40°C was added until the volume in the flask was 105 ml. the

sample was then vigorously agitated and filtered. Twenty-five Milliliters of the filtrate were pipetted into a porcelain dish, 5 drops of phenolphthalein were added, and the sample was titrated against 0.1N NaOH till a faint pink colour that lasted for at least 30 seconds was obtained. The acidity was calculated fro the following equation:

$$\text{Titratable acidity} = \frac{T}{W}$$

Where T= Titration figures W= Weight of samples

3.2.1.2Protein content:-

Protein content was determined by Kjeldhal method; according to AOAC (2009) three grams of the cheese and two Kjeldhal tablets (1gramNa_2So_4 and equivalent of 0.1 gram Hg) were put into Kjeldhal flask. Twenty-five ml of concentrated sulphuric acid (density of 1.86 mg/ml at 20°C) were added to the flask. The mixture was then digested on a heater until a clean solution was obtained (3hours) and the flask were removed and left to cool. The digested sample was poured into a volumetric flask (100 ml) and diluted to 100 ml with distilled water. The distillate was received in a conical flask containing 25 ml of 4% boric acid plus three drops of indicator (bromerol green plus methyl red). The distillation was continued until the volume in the flask was 75 ml. The flask was then removed from the distillatory, the distillate was then titrated against 0.1NHCl until the end point was obtained (red colour).

Protein content was calculated as follows:

$$\text{Nitrogen \%} = \frac{T \times 0.1 \times 20 \times 0.014 \times 100}{\text{Weight of sample}}$$

Protein %= Nitrogen % X 6.38

Where:

T = Titration figure

0.1 N = Normality of HCl

0.014 = The atomic weight of nitrogen/1000

20 = Dilution factor

3.2.1.3 Fat content:-

Fat was determined by Gerber's method according to AOAC (2009). In a clean dry cheese Gerber tube, 10 ml of sulphuric acid (density1.815 gram/ml at 20°C) were poured, and then 3 grams of minced cheese samples were added. Amyl alcohol (1 ml) was added to the mixture followed by the addition of distilled water. The contents were thoroughly mixed till no white particles could be seen. The Gerber tubes were centrifuged at 110 revolutions per minutes for five minutes and the tubes were then transferred to water bath adjusted at 65°C for three minutes. The fat percent was then read out directly from the fat column.

3.2.1.4Ash content:-

Ash content was determined according to the method of AOAC (2009). Two grams of cheese sample were weighed in a suitable clean and dry crucible and evaporated to dryness on a steam bath. Then the sample was placed in a muffle furnace at 550°C for 2 hours, and then cooled in a desiccator and weight.

The ash content was calculated using the following equation:

$$\text{Ash \%} = \frac{W_1}{W_0} \times 100$$

Where:

W_1 = weight of ash.

W_0 = weight of sample.

3.2.1.5 Total solids content:-

Total solids were determined according to the modified methods of AOAC (2009). Two grams of cheese sample were weighted and placed a clean dried porcelain dish. The weight of sample and dish were recorded and the dishes were heated on a steam bath for 10-15 minutes. The dishes were then placed in an oven at 100°C for three hours, after which they were transferred to dessicator to cool and then weighted. Heating, cooling and weighting were repeated several times until the difference between successive weighing was less than 0.1 gram.

Total solids content were calculated from the following equation:

$$TS\% = \frac{W_1}{W_0} \times 100$$

Where:

W_1 = weight of sample after drying.

W_0 = weight of sample before drying.

3.2.1.6 Volatile fatty acids content (VFA):-

Total volatile fatty acids contents of the cheese samples were determined by the direct distillation method of Kosikowski (1982). Ten grams of cheese were placed in a mortar and grounded with successive portions of 10% sulfuric acid until the cheese was completely emulsified then transferred to 500 ml kjeldahl flask. The addition continued until the volume of the acid added to the sample reached 25 ml. About 17.5 grams of magnesium sulphate were added to the contents in the flask, followed by few glass beads, 250 ml of distilled water and the contents distilled. Distillation was terminated when 280 ml of the distillate were collected. The inside tube of the condenser was rinsed with 12.5 ml of neutral alcohol to remove the insoluble volatile acids combined with distillate and titrated with 0.1 N NaOH. The total volatile fatty acids contents were expressed as ml of 0.1 N NaOH that neutralized the distillate from 100 grams of cheese.

3.2.1.7 Calcium, phosphorus and Potassium determination:-

Calcium, phosphorus and Potassium contents of the samples were determined by Atomic Absorption Spectrometer according to Perkin (1994) and AOAC

(2009).Two grams of cheese was maintained in muffle furnace at 550^{o}C for 4 hours. Samples were cooled and 10ml of 3NHCL was added , covered with watch glass and boiled gently for 10 minutes. Then cooled ,filtered , diluted to volume (100ml) with distilled water and taken for determination of phosphorus (P) Calcium (C) potassium (K) and for the determination of calcium 1 ml 0f 1% Lanthanum chloride was added to the final dilution .

Mg mineral /100 mg sample $= \frac{\text{mg/L volum used} \times 100}{1000 \times \text{wt.of sample}}$

3.3.1 Microbiological analysis:-

Samples were examined for total bacteria count (TBC), yeast and moulds, *Staphylococcus aureus*, Coliforms, *Escherichia coli*, Salmonella and *Listeria monocytogens.*

3.3.2 Preparation of media and glassware:-

3.3.2.1 Sterilization of glassware:-

Petri dishes, test tubes, flasks pipettes, etc. were sterilized in a hot air oven at 160-180°C for 2- 3 hours. Before they were put in the oven, they were washed, dried and packed in stainless steel cans. Instruments such as loops, needles, forceps and knives were sterilized by flaming directly after dipping in spirit (Harrigan, 1998) .

3.3.2.2 Sterilization of media:-

Culture media were first adjusted to the PH and then sterilized. Sterilization was achieved by autoclaving at 121°C for 15minutes(Harrigan, 1998).

3.3.3 Total viable count of bacteria (TBC):-

Total viable count for bacteria was carried out using the standard plates count method as described by (Harrigan, 1998) suitable medium for this purpose is Plate count agar.

3.3.3.1 Preparation of serial dilutions:-

Aseptically 10 gram of the sample were homogenized in 90ml, of dilution (0.1% peptone solution). It was mixed well to give dilution (10^{-1}) by using sterile pipettes 1ml was transferred aseptically from dilution (10^{-1}) to a test tube containing 90ml, of sterile dilution and it was mixed well 3e44to give dilution (10^{-2}) .In the same way preparation of sterile dilution was continued until the dilution (10^{-5}) .One ml of each dilution was transferred aseptically into sterile Petri dishes plate count agar was added. The inoculums were mixed with medium and allowed to solidify. The plates were then incubated at 37°C for 48 hours. A colony counter (Quebec Colony Counter and Hand Tally) was used to count the viable bacterial colonies after incubation and the results were expressed as colony forming units per gram (cfu/ml).

3.3.4 Determination of *coliform* bacteria:-

Coliform count was carried out using the Most Probable Number (MPN) technique.

3.3.4.1 Presumptive *coliforms* tests:-

Nine tubes each containing nine ml of MacConkey broth (enrichment medium), fitted with Durham tubes were used. One ml from suitable dilutions

of cheese samples of each of first three dilutions (10^{-1}, 10^{-2}, 10^{-3}) were incubated in triplicates of MacConkey broth test tubes containing Durham tubes. The tubes were incubated at 37^{o}C for 48 hours. The Growth and gas production after 24 and 28 hrs were recorded .Gas production constituted a positive test (Harrigan ,1998).

3.3.4.2 Confirmed *coliforms* tests:-

The positive tubes of MacConkey broth that showed acid and gas formation were agitated and one loopful from each positive sample was transferred to a tube of Brilliant green lactose bile broth (BGB). The BGB broth tubes were incubated at 37^{o}C for 24 – 48 hours. The tube which shows gas in the inverted Durham tubes and turbidity of the medium were recorded as positive for confirmation of total coliforms. Coliforms most probable number per ml (MPN/ml) was calculated from the number of positive MacConkey broth tubes in the presumptive tests using special tables and formula for the purpose (Harrigan ,1998).

3.3.5 *E. coli* counts:-

The medium used in this test was EC broth (*Escherichia coli* media). From every tube showing positive result in the presumptive test incubated a test tube of EC broth containing Durham tubes. The tubes were incubated at 44.5^{o}C for 24 hours.

Tubes showing any amount of gas were considered positive then the Most Probable Number (MPN) was recorded for further confirmation of tubes of EC showing positive result at44.5^{o}C for 24 hours were streaked on Eosin Methylene Blue (EMB) agar plates. The plates were incubated at 37^{o}C for 48

hours. Colonies of *E. coli* with green metallic shine gave a positive test (Harrigan, 1998).

3.3.6 *Staphylococcus aureus* count:-

From suitable dilutions of cheese samples, 0.1ml was aseptically transferred into sterile Petri dish containing Baird-parker medium. The inoculums were spread all over the plates using sterile bent glass rod. Plates were then incubated at 37°C for 24 hours. After the period of incubation had been finished the plates.

Staphylococcus aureus after 24hours appear were examined black shiny convex and surrounded by a zone of clearing 2-5 mm in width of colony (Harrigan,1998).

3.3.7 *Salmonella*counts:-

Ten grams of cheese samples mixed well with 100 ml sterile nutrient broth, then incubated at 37°C for 24 hours. Then 10 ml were aseptically drawn and added to 100 ml sterile broth. The broth was incubated at 37°C for 24 hours. A loop full of 24 hours incubated was transferred aseptically into sterilized selenite cystine broth and incubated at 37°C for 24 hours. Using a loop full, streaking was carried out into solidified Bismuth sulphite agar plates. The plates were incubated at 37°C for 24-72 hours.

Black metallic shine discrete colonies indicated the presence of *Salmonella*. A confirmatory test was carried out by taking a discrete black sheen colonies and sub culturing it in triple sugar iron agar tubes. After incubation the production

of black color at the bottom of the tube confirmed the presence of *Salmonella* (Harrigan, 1998).

3.3.8 *Listeria monocytogens*:-

Ten grams cheese were weighed aseptically and added to 100 ml Fraser both base to form 10^{-1}dilution and mixed with a stomacher.

A decimal dilution series was prepared in 0.1% peptone solution in the usual way. The Surface inoculate 0,1 ml amounts of dilutions on to prepared predried plates of PALCAM and oxford agar. The plates were incubated at 37◦c for 48 hours (the oxford agar plates aerobically and the PALCAM agar plates under the micro aerobic condition. The Colonies of listeria on PALCAM agar are green and have black haloes they may also have black center.*Listeria monocytogens* on oxford agar Colonies with dark brown or black haloes due to an esculin hydrolysis (Harrigan, 1998).

3.3.9 *Yeast* and *mould* counts:-

Sabouraud dextrose agar was used for enumeration of yeasts and moulds. For isolation 0.2 ml portions of each samples decimal dilution 10^3 and 10^4 were streaked in duplicates on the dried medium. The plates were incubated at 30^oC for three days. After 3 days colonies of yeasts and moulds were counted by colony counter and recorded. Total yeasts and moulds were calculated as the number of colony forming units per ml of samples (Harrigan and McCance, 1976). *Yeasts* cultures were also identified by microscopic examination using Gram's stain method.

3.4 Statistical analysis:-

Statistical analysis was done using SPSS programme (1998). General linear model was used to estimate the effect of area and packing materials and interactions between them on the chemical and microbiological quality of White, Romi and Mozzarella cheese also descriptive analysis was done for the analysis of microbial contents of the cheese samples. Least Significant Difference was used for means separation between the treatments. The levels of significance $\alpha<0.05$ was used in this study.

CHAPTER FOUR

Results

4.1 Quality of white cheese:-

4.1.1 Chemical composition of white cheese:-

Data in table (1) showed the effect of packaging materials on the chemical composition of the white cheese samples. The result indicated that the fat content of white cheese samples was significantly (P< 0.01) affected by the packing materials. The highest value (24.23 ± 1.10 %) was found in the white cheese samples in plastic pack. and the lowest one (21.74 ± 1.46 %) was obtained by the cheese samples in the paper package.

Protein content of white cheese samples was significantly (P< 0.001) affected by the packaging materials. The highest value (14.62 ± 0.98 %) was in the white cheese samples in plastic pack, while the lowest one (13.89 ± 0 .97 %) was recorded by that in the paper pack.

Total solids of white cheese samples was significantly (P< 0.01) affected by the packaging materials. The highest total solids content (50.16 ± 2.37 %) was for the white cheese samples in plastic pack, however, the lowest value (41.51 ± 2.70 %) was found in the paper package.

Volatile fatty acids of white cheese samples were significantly (P< 0.01) affected by the packing materials. The highest value (7.06 ± 0 .21 ml of 0.1 N

NaOH/100 g cheese) was found in the white cheese samples in the paper package, and the lowest one (5.44 ± 0.74ml of 0.1 N NaOH/100 g cheese) was recorded by the one in plastic pack.

Results revealed that titratable acidity of the white cheese samples was significantly (P< 0.001) higher (0.96± 0.10 %) in cheese samples packed in plastic than that in paper pack (0.85 ± 0 .24 %).

Ash content of white cheese samples was significantly (P< 0.001) affected by the packaging materials. The highest value (5.84 ± 1.15 %) was found in white cheese samples in plastic pack, and the lowest one (3.72 ± 0.55 %) was in that in paper pack.

Calcium, Phosphorus and Potassium contents of the white cheese samples were significantly (P< 0.00) affected by the packing materials. The highest values (0.60 ± 0.14 %, 0.37 ± 0.07 % and 0.22 ± 0.06 %) respectively for the above mentioned mineral were found in white cheese samples in in plastic pack.

Table (1): The effect of packing materials on the chemical composition of white cheese in Khartoum State:

Chemical Composition									
Packing	**Fat %**	**protein %**	**Total solids %**	**VFA ml 0.1 N NaOH/100g cheese**	**Titra.acidity %**	**Ash %**	**Ca %**	**P %**	**K %**
Plastic	24.2 ± 1.10 [a]	14.62±0.98[a]	50.16 ±2.37[a]	5.44 ± 0.74[b]	0.96 ± 0 .24 [b]	5.84 ± 1.15[a]	0.60 ±0.14 [a]	0.37± 0.07 [a]	0.22± 0.06 [a]
Paper	21.74±1.46 [b]	13.89±0.97[b]	41.51± 2.70[b]	7.06 ± 0.21[a]	0.85 ± 0.10[a]	3.72 ± .55[b]	0.4 ± 0.09[b]	0.23 ± 0.07[b]	0.19 ± 0.09 [b]
Level of sig.	**								

Mean values bearing different superscripts within columns are significantly different (P< 0.05).

Results in table (2) demonstrated the chemical composition of white cheese samples in different areas in Khartoum state. The result indicated that different area was not significantly (P>0.05) affected the fat content of white cheese samples. The highest fat content (23.03±1.69 %) was secured by the white cheese samples in Khartoum north and the lowest one (22.98±1.86%), was found in the white cheese samples from Khartoum.

Total solids content of white cheese sample was not significantly (P>0.05) affected by the different area. The highest value (46.22 ± 4.96%) was found in white cheese samples from Khartoum and the lowest one (45.35 ± 4.51%) was recorded by that Khartoum north.

Volatile fatty acids of white cheese samples were not significantly affected (P>0.05) by the different areas.

The data showed that titratable acidity of white cheese samples was not significantly (P>0.05) affected by the different area. The highest value (0.79 ± 0.18 %) was noted by the white cheese sample from Khartoum the lowest one (0.76 ± 0.20%) was obtained by the white cheese samples Omdurman.

Ash, Calcium, Phosphorus and Potassium contents of white cheese samples were not significantly (P>0.05) affected by the different area.

Table (2): Effect of area on the chemical composition of white cheese in Khartoum:

Area	Chemical composition								
	Fat %	**protein %**	**Total solids %**	**VFA ml 0.1 N NaOH/g cheese**	**Titra.acidity %**	**Ash %**	**Ca %**	**P %**	**K %**
Khartoum	22.98±1.86	14.47±0.91	46.22 ± 4.96	6.22 ± 1.07	0.79 ±0 .18	4.70±1.46	0.50 ± 0.16	0.32 ± 0.10	0.19 ± 0.07
Khartoum North	23.03±1.69	14.08±1.17	45.35 ± 4.51	6.29 ±0 .91	0.77 ± 0.22	4.65±1.43	0.49 ± 0.14	0.27 ± 0.13	0.19 ± 0.09
Omdurman	22.90±1.89	14.20±1.02	45.73 ± 5.63	6.27 ±0 .96	0.76 ± 0.20	4.94±1.29	0.54 ± 0.14	0.31 ± 0.07	0.23 ± 0.07
Lev. of sig.	NS								

Mean values bearing different superscripts within columns are significantly different (P< 0.05).

Data in table (3) showed the effect of interaction between the area and packaging materials on the chemical composition of white cheese samples. The result indicated that all the chemical parameters except the Total solids were not significantly different P>0.05) in different areas. The high fat (24.45 ±0.94 %) was for the white cheese samples in plastic pack from Khartoum, while the lowest one (21.50 ±1.28 %) was found in paper pack from Khartoum.

The higher protein content (14.77 ±0.86 %) was in cheese samples in plastic package from Khartoum, and the lowest one (14.18 ±0.87 %) was for the white cheese samples in paper pack from Khartoum.

The highest VFA (7.09±0.18 %) was in the white cheese samples in paper package from Khartoum, the lowest one (5.35±0.85 %) was in plastic package from Khartoum.

The highest titratable acidity (0.83±0.09 %) was obtained by the white cheese sample in paper pack from Khartoum, and the lowest one (0.75±0.23 %) was in that in plastic pack from Khartoum.

Highest contents (5.86±1.14%) were obtained by the white cheese samples in plastic pack from Khartoum, while the lowest one (3.55 ±0.48%) was in paper package from Khartoum.

The highest calcium content (0.60±0.16 %) was recorded in the white cheese samples in plastic pack from Khartoum, the lowest one (0.40±0.10 %) was for that in paper pack from Khartoum.

The highest Phosphorous value (0.40±0.07 %) was in white cheese samples in plastic pack from Khartoum, the lowest one (0.24±0.05 %) was found in that in paper pack from Khartoum.

The highest Potassium content (0.20 ±0.07 %) was found in the white cheese samples in plastic pack from Khartoum, and the lowest one (0.18 ±0.08 %) was in paper pack from Khartoum.

Total solids of the white cheese samples was affected significantly (P>0.01) by the area and packing materials. The highest Total solids value (50.68± 2.26 %) was found in the white cheese samples from Khartoum in plastic pack, the lowest one (41.77±1.90 %) was for that in paper pack from Khartoum.

Table (3): Effect of interaction between the area and packing materials on the chemical composition of white cheese in Khartoum state:

Area	Chemical composition									
	Pack.	**Fat %**	**protein %**	**Total solids %**	**VFA ml 0.1N NaOH/100 g cheese**	**Titra.acidity %**	**Ash %**	**Ca %**	**P %**	**K %**
Khartoum	**Plastic**	24.45 ±0.94	14.77 ±0.86	50.68±2.26	5.35±0.85	0.75±0.23	5.86±1.14	0.60±0.16	0.40±0.07	0.20 ±0.07
	Paper	21.50 ±1.28	14.18 ±0.87	41.77±1.90	7.09±0.18	0.83±0.09	3.55 ±0.48	0.40±0.10	0.24±0.05	0.18 ±0.08
Khartoum North	**Plastic**	24.16 ±0.90	14.56 ±0.95	49.06±2.27	5.47±0.62	0.65± 0.25	5.71 ±1.23	0.56±0.15	0.36±0.09	0.22 ±0.04
	Paper	22.00±1.58	13.65 ±1.21	41.90±3.18	7.03±0.23	0.87±0.12	3.69 ± 0.76	0.42±0.08	0.18±0.08	0.16 ±0.06
Omdurman	**Plastic**	24.10 ±1.41	14.55 ±1.15	50.69±2.33	5.50±0.76	0.68±0.25	5.94 ±1.12	0.64±0.11	0.36±0.05	0.24 ±0.05
	Paper	21.70 ±1.53	13.86 ±0.74	40.77±2.82	7.05±0.21	0.83±0.08	3.93 ±0.17	0.44±0.09	0.26±0.05	0.22 ±0.08
Lev. Sig.		NS		*	NS					

4.1.2. Microbiological composition of white cheese:-

Table (4) showed the effect of total bacterial count (Log colony forming nit/ml) of white cheese samples was not significantly (P>0.05) by the packing materials. The highest counts (5.47 ± 0.76 log cfu/ml) was obtained by the white cheese samples were packed in paper pack, the lowest count (5.37 ± 0.71 log cfu/ml) was found in the one in plastic pack .

Table (5) showed the effect of total bacterial count (Log colony forming unit/ml) of white cheese samples was not significantly (P>0.05) by the area. The highest counts (5.48 ± 0.70 log cfu/ml) was obtained by the samples of white cheese were selected from Khartoum north ,the lowest counts (5.39 ± 0.79 cfu/ml , 5.39 ± 0.71 cfu/ml) were recorded by the white cheese sample in paper pack were selected from Omdurman and Khartoum respectively.

Table (6) showed the effect the different area and packing materials on the total bacterial count (Log colony forming unit/ml) of white cheese samples. No significant variations (P>0.05) were noted in the microbial contents. The highest TBC count (5.40 ± 0.79 log cfu/ml) was for the white cheese sample from Khartoum in paper package, the lowest count (5.39 ± 0.64 cfu/ml) was for the one in plastic pack from Khartoum.

Table (4): Effect of packing materials on Total bacterial count of White cheese in Khartoum State:

Total bacterial count (log cfu/ml)	
Packing	**TBC**
Plastic	5.37 ± 0.71
Paper	5.47 ± 0.76
Level of sig.	NS

Mean values bearing different superscripts within column are significantly different (P< 0.05).

Table (5): Effect of area on total bacterial count of white cheese in Khartoum State:

Total bacterial count (log cfu/ml)	
Area	**TBC**
Khartoum	5.39 ± 0.71
Khartoum North	5.48 ± 0.70
Omdurman	5.39 ± 0.79
Level of sig.	NS

Mean values bearing different superscripts within column are significantly different (P< 0.05).

Table (6): Effect of interaction between the area and packing materials on the total bacterial count of white cheese in Khartoum State:

Total bacterial count (log cfu/ml)		
Area	**Packing**	**TBC**
Khartoum	**Plastic**	5.39 ± 0.64
	Paper	5.40 ± 0.79
Khartoum North	**Plastic**	5.34 ± 0.66
	Paper	5.61 ± 0.73
Omdurman	**plastic**	5.38 ± 0.82
	paper	5.41 ± 0.76
Level of sig.		NS

Mean values bearing different superscripts within column are significantly different (P< 0.05).

Table (7) showed the microbial load in white cheese samples in different packing materials in Khartoum state. The result indicated that the total presence of yeast and molds in white cheese samples in Khartoum state was (7.5%) with count range (2.4 – 2.7 log cfu/ml). The high percent was found in white cheese samples in plastic pack in Khartoum.

Staphylococcus aureus was found only in (4%) of the white cheese samples in Khartoum state with count ranged between (2.5 - 2.8 Lg cfu/ml). The highest percent (10%) of *Staphylococcus aureus* was found in white cheese samples in plastic pack from Khartoum. and the lowest one (5%) was noted by the white cheese samples in paper pack.

Coliforms were found only in (0.83%), of white cheese samples in Khartoum state with count ranged between (0.0 - 3.0 Log cfu/ml).

Coliforms were determined in white cheese samples (5%) in plastic pack from Omdurman. and was not found in paper pack in all cheese samples

Escherichia coli , *Salmonella* . *spp* and *Listeria monocytogens* were not detected in all white cheese samples.

Table (7): Microbiological composition (%) of white cheese samples in Khartoum state:

Microbial contents	Packaging	Area			Microbial log cfu/ml
		Khartoum	Khartoum North	Omdurman	
Yeast and mold	Plastic	15	5	10	2.5 – 2.7
	Paper	5	5	5	2.4 – 2.6
	Total	7.5			2.4 – 2.7
Staph. Areus	Plastic	10	5	5	2.5 – 2.8
	Paper	5	ND	ND	0.00 – 2.5
	Total	4			2.5 – 2.8
E. coli	Plastic	ND	ND	ND	ND
	Paper	ND	ND	ND	ND
	Total	ND			ND
Coliforms	Plastic	ND	ND	5	0.00 – 3.00
	Paper	ND	ND	ND	ND
	Total	0.83			0.00 – 3.00

ND= Not Detected

4.2Quality of Romi cheese:-

4.2.1chemical composition of Romi cheese:-

Data in table (8) showed the effect of packaging materials on the chemical composition of Romi cheese samples. The result demonstrated that fat content of Romi cheese samples was significantly affected (P< 0.01) by the packing materials. The highest fat value (29.40±1.41%) was obtained by the Romi cheese samples in paper pack, and the lowest one (23.31±1.88%) was found in the one in plastic pack.

Protein content of Romi cheese samples was significantly affected (P< 0.01) by the packing materials. The highest value (25.76±2.04%) was recorded by the Romi cheese samples in paper pack, and the lowest one (16.77±1.26%) was found in Romi cheese samples in in plastic pack.

Total solids content of Romi cheese samples was significantly affected (P< 0.01) by the packing materials. The highest Total solids value (64.40±4.12%) was recorded by the Romi cheese in paper pack, and the lowest one (48.86±2.37%) was one in Romi in plastic pack.

Volatile fatty acids of Romi cheese samples were affected significantly (P< 0.01) by the packing materials. The highest value (11.42±1.43 ml 0.1N NaOH/100 g cheese) was noted by the Romi cheese samples in paper pack, and the lowest one (6.14±0.58 ml 0.1N NaOH/100 g cheese) was found in the one in plastic pack.

Titratable acidity of Romi cheese samples was significantly affected (P< 0.001) by the packing materials. The highest value (0.99±0.25%) was recorded by the Romi cheese samples in plastic pack, and the lowest one (0.83±0.19%) in the one in paper pack.

Ash content of Romi cheese samples were significantly affected (P< 0.001) by the packing material. The highest value (4.45±0.80%) was found in Romi cheese samples on paper pack, and the lowest one (4.02±0.47%) was noted by the one Romi in plastic pack.

Calcium, Phosphorus and Potassium contents of Romi cheese samples were affected significantly (P< 0.01) by the packing materials. The highest values were; (0.85±0.12%); (0.90±.05%) and (0.25±0.06%) for Calcium, Phosphorus and Potassium contents respectively of the Romi cheese samples in paper pack, plastic pack, and in plastic pack respectively.

Results in table (9) showed the chemical composition of Romi cheese samples in different areas. The result showed that fat, VFA and the ash contents of the Romi cheese samples were significantly (P<0.01) varied in different areas. The highest fat content (26.85±3.44 ml 0.1N NaOH/100 g cheese) was in the Romi cheese samples from Omdurman and the lowest one (26.45±3.13% ml 0.1N NaOH/100 g cheese) was in that from Khartoum The highest VFA content (9.18±3.21 ml 0.1N NaOH/100 g cheese) was obtained by the Romi cheese samples from Khartoum North and the lowest one (8.79±2.87 ml 0.1N NaOH/100 g cheese) was also from Khartoum. The highest ash value (4.44±0.58 ml 0.1N NaOH/100 g cheese) was obtained by the Romi cheese samples from Omdurman and the lowest one (4.24±0.57 ml 0.1N NaOH/100 g cheese was from Khartoum.

Table (8): Effect of packing materials on the chemical composition of Romi cheese in Khartoum State:

Chemical Composition									
Package	**Fat %**	**Crude protein %**	**Total solids %**	**VFA ml 0.1N NaOH/100 g cheese**	**Titra.acidity %**	**Ash %**	**Ca %**	**P %**	**K %**
Plastic	23.31±1.88[b]	16.77±1.26[b]	48.86±2.37[b]	6.14±0.58[b]	0.99±0.25[a]	4.02±0.47[b]	0.29±0.04[b]	0.90±.05[a]	0.25±0.06[a]
Paper	29.40±1.41[a]	25.76±2.04[a]	64.40±4.12[a]	11.42±1.43[a]	0.83±0.19[b]	4.45±0.80[a]	0.85±0.12[a]	0.71±.14[b]	0.15±0.05[b]
Lev. sig.	***								

Mean values bearing different superscripts within columns are significantly different (P< 0.05).

Protein, total solids, titratable acidity, Ca, P and K were not significantly (P>0.05) different in the Romi cheese from different areas. The highest protein content (21.66±4.90%) was found in Romi cheese samples from Omdurman and the lowest one (21.30±4.79%) was for the Romi cheese samples from Khartoum .The highest total solids content (57.02±8.23%) was noted by Romi cheese samples from Khartoum and the lowest value (56.87±8.57%) was found in one from Khartoum north. The highest titratable acidity value (0.92±0.28%) was for the Romi cheese samples from Khartoum north and the lowest one (0.91±0.21%) was found in that from Omdurman.

The results in table (10) explained the chemical composition of Romi cheese as affected by the packaging materials in different areas. The result indicated that no significant variations were observed in the chemical composition of the Romi cheese samples due to the interaction between the area and packaging type except the VFA and the titratable acidity.

4.2.2 Microbiological composition of Romi cheese: -

Data in table (11) showed total bacterial count (Log colony forming unit/ml) of the Romi cheese samples in different packages in Khartoum state, statistical analysis showed that no significant (P>0.05) difference was found in the total bacterial count of the Romi cheese samples in different packages. The higher total bacterial counts (5.43 ± 0.67 cfu/ml) were in the Romi cheese samples in paper pack in comparison to the plastic pack.

Table (12) and table (13) showed the total bacterial count (Log colony forming unit/ml) of the Romi cheese samples in different packages and different areas. Also no significant variations were observed in the total bacterial count.

Table (9): The chemical composition of Romi cheese in different areas in Khartoum State:

Area	Chemical composition								
	Fat %	**Crude protein %**	**Total solids %**	**VFA ml 0.1N NaOH/100 g cheese**	**Titra.acidity %**	**Ash %**	**Ca %**	**P %**	**K %**
Khartoum	26.08±3.86^{cb}	21.29±4.90	57.02±8.23	8.62±2.53^{cb}	0.89±0.22	4.24±0.57^{ba}	0.57±0.29	0.78±0.16	0.20±0.08
Khartoum North	26.45±3.13^{ba}	21.30±4.79	56.87±8.57	9.18±3.921^{a}	0.92±0.28	4.08±0.86^{cb}	0.57±0.33	0.82±0.15	0.20±0.09
Omdurman	26.85±3.44 ^{a}	21.66±4.90	56.78±8.90	8.79±2.87^{ba}	0.91±0.21	4.44±0.58^{a}	0.56±0.30	0.82±0.11	0.21±0.06
Level of sig.	***	NS		**	NS	*	NS		

Mean values bearing different superscripts within columns are significantly different (P< 0.05).

Table (10): Interaction between the area and packing materials on the chemical composition of Romi chee

Area	Chemical composition							
	Packing	Fat %	protein %	Total solids %	VFA ml 0.1N NaOH/100 g cheese	Titra.acidity %	Ash %	Ca %
Khartoum	**Plastic**	22.39±1.85	16.59±1.14	49.48±2.41	6.19±0.47	0.68±0.09	3.97±0.40	0.30±0.01
	Paper	29.09±1.93	25.14±3.02	63.19±5.71	10.61±1.58	1.06±0.12	4.45±0.61	0.84±0.11
Khartoum North	**Plastic**	23.80±2.19	16.72±1.36	48.74±2.35	6.15±0.60	1.15±0.19	3.90±0.58	0.28±0.04
	Paper	29.10±0.72	25.87±1.09	64.10±2.45	12.22±1.17	0.69±0.09	4.27±1.05	0.86±0.17
Omdurman	**Plastic**	23.65±1.27	16.97±1.28	48.41±2.35	6.08±0.67	1.10±0.09	4.19±0.35	0.28±0.04
	Paper	30.05±1.05	26.35±1.13	65.14±3.14	11.50±0.98	0.72±0.06	4.69±0.66	0.84±0.09
Lev. Sig.		NS			***		N	

Table (11): Effect of packing materials on total bacterial count of Romi cheese in Khartoum state:

Package type	TBC log cfu/ml
Plastic	5.37 ± 0.62
Paper	5.43 ± 0.67
Level of sig.	NS

Mean values bearing different superscripts within columns are significantly different (P< 0.05).

Table (12): Effect of area on total bacterial count of Romi cheese in Khartoum State:

Area	Total bacterial count TBC (log cfu/ml)
Khartoum	5.31 ± 0.65
Khartoum North	5.41 ± 0.63
Omdurman	5.48 ± 0.66
Level of sig.	NS

Mean values bearing different superscripts within columns are significantly different (P< 0.05).

Table (13): Interaction between the area and Packing materials on total bacterial count of Romi cheese in Khartoum State:

Total bacterial count (log cfu/ml)		
Area	**Packing**	**TBC**
Khartoum	**Plastic**	5.14 ± 0.48
	Paper	5.49 ± 0.74
Khartoum North	**Plastic**	5.44 ± 0.62
	Paper	5.38 ± 0.65
Omdurman	**Plastic**	5.55 ± 0.69
	Paper	5.42 ± 0.64
Level of sig.		NS

Mean values bearing different superscripts within rows are significantly different (P< 0.05).

Table (14) showed the microbial load of Romi cheese samples in different packing materials in Khartoum state. The result revealed that Overall, 9 % of studied samples were found to be contaminated with yeasts and moulds of Romi cheese samples in Khartoum state with count ranged between(2.4 _ 2.7 Lg cfu/ml). The highest percent (15%) count was found in the Romi cheese samples in plastic pack, the lowest one (5%) was noted by the Romi cheese samples in paper pack.

Staphylococcus aureus was only in (2.5%) of Romi cheese samples in Khartoum state with count ranged between (2.5 _ 2.8 Lg cfu/ml). Five percent of Romi cheese samples in plastic and paper pack in Khartoum were contaminated with *S. aureus* , however not detection of this bacteria in Romi cheese samples in Khartoum North and only in those in paper pack in Omdurman.

Coliforms counts were found only in (1.6%), of Romi cheese samples in Khartoum state with the count ranged between (3.0 _ 3.0 Lg cfu/ml). *Coliforms* were not determined in Romi cheese samples in Omdurman, the highest percent (5%) were found in Romi cheese samples in paper pack in Khartoum and Khartoum North once not detected in that in plastic pack.

Romi cheese samples in Khartoum state was contaminated with *Escherichia coli* (0.83%) the count ranged between (3.0 _ 0.00 Lg cfu/ml). the highest percent (5%) was found in Romi cheese samples in paper pack in Khartoum North, while was not detected in that in Khartoum and Omdurman. *Salmonella spp* and *Listeria monocytogens* were not detected in Romi cheese samples.

Table (14): Microbiological composition of Romi cheese samples in Khartoum state:

Microbial contents	Packaging	Area			Microbial log cfu/ml
		Khartoum	Khartoum North	Omdurman	
Yeast and mold	Plastic	15	10	10	2.4 – 2.6
	Paper	10	5	5	2.5 – 2.7
	Total	9			2.4 – 2.7
Staph. Areus	Plastic	5	ND	ND	0.0 - 2.8
	Paper	5	ND	5	2.5 – 2.6
	Total	2.5			2.5 – 2.8
E. coli	Plastic	ND	ND	ND	ND
	Paper	ND	5	ND	0.00 – 3.00
	Total	0.83			0.00 – 3.00
Coliforms	Plastic	ND	ND	ND	ND
	Paper	5	5	ND	3.00 – 3.00
	Total	1.6			

ND= Not detected

4.3. Quality of Mozzarella cheese:-

4.3.1Chemical composition of Mozzarella cheese:-

In this study Mozzarella cheese was found to be packed only in plastic pack. Table (15) showed the chemical composition of Mozzarella cheese in plastic pack from different area.

The results of statistical analysis demonstrated that only Total solids, VFA and titratable acidity of the Mozzarella cheese samples in Khartoum state were significantly($P< 0.01$) different in the different areas. The higher values (65.14±3.14 %; 11.50±0.98 % and 0.72±0.06 %) for Total solids, VFA and titratable acidity of Mozzarella cheese samples from Omdurman area in comparison with those in the other areas. However, no significant differences were observed in the fat, protein, ash, Ca, P and K of the Mozzarella cheese samples in the different areas.

Table (15): Chemical composition of Mozzarella cheese in plastic pack in Khartoum State:

Area	Chemical composition								
	Fat %	**protein %**	**Total solids %**	**VFA ml 0.1N NaOH/100 g cheese**	**Titra.acidity %**	**Ash %**	**Ca %**	**P %**	**K %**
Khartoum	20.05±1.15	15.37±1.88	52.23±5.79^{cb}	4.74±0.59^{cb}	0.38±0.04^{b}	4.51±0.60	0.76±0.05	0.90±0.10	0.12±0.04
Khartoum North	19.75±1.07	15.50±2.30	52.81±6.51^{b}	4.84±0.63^{b}	0.37±0.05^{cb}	4.27±1.05	0.72±0.04	0.90±0.01	0.14±0.05
Omdurman	20.00±.97	14.63±2.42	65.14±3.14^{a}	11.50±0.98^{a}	0.72±0.06^{a}	4.69±0.66	0.78±0.04	0.88±0.04	0.16±0.05
Lev. Sig.	NS		***			NS			

Mean values bearing different superscripts within columns are significantly different (P< 0.05).

4.3.2Microbiological composition of Mozzarella cheese:-

Data in table (16) showed the total bacterial count (Log colony forming unit/ml) of Mozzarella cheese samples in Khartoum stated. The statistical analysis revealed no significant (P>0.05) variations were observed in the total bacterial count in the Mozzarella cheese samples in different areas. The higher TBC counts (5.24 ± 0.65 cfu/ml) were obtained by the Mozzarella cheese samples from Khartoum in comparison with other samples.

Table (17) described the microbial load of Mozzarella cheese samples in Khartoum state. The result indicated that yeasts and molds were found only in (6.6%) of Mozzarella cheese samples in Khartoum state with count ranged between (2.5 _ 2.8 Lg cfu/ml), the highest count (10%) was for the Mozzarella cheese samples from Khartoum, and the lowest one (5%),was in that from other areas .

Staphylococcus aureus were found only in (1.6%) of Mozzarella cheese samples in Khartoum state with count ranged between (0.0 _ 2.5 Lg cfu/ml). The presence of only (5%) *S.aureus* in the Mozzarella cheese samples from Khartoum and was not detected in Khartoum north and Omdurman areas .

Coliforms, Escherichia coli, Salmonella . spp and *Listeria monocytogens* were not detected in all Mozzarella cheese samples in Khartoum state.

Table (16): The total bacterial count of Mozzarella cheese in plastic pack in Khartoum State:

Total bacterial count (log cfu/ml)	
Area	**TBC**
Khartoum	5.24 ± 0.65
Khartoum North	5.18 ± 0.66
Omdurman	4.90 ± 0.40
Level of sig.	N.S

Mean values bearing different superscripts within columns are significantly different (P< 0.05).

Table (17): Microbiological contents of Mozzarella cheese samples in plastic package in Khartoum state:

Microbial contents	Packaging	Area			Total (%)	Microbial log cfu/ml
		Khartoum	Khartoum North	Omdurman		
Yeast and mold	**Plastic**	10	5	5	6.6	2.5 _ 2.8
Staph. Areus	**Plastic**	5	ND	ND	1.6	0.0 _ 2.5
E. coli	**Plastic**	ND	ND	ND	ND	ND
Coliforms	**Plastic**	ND	ND	ND	ND	ND

ND= Not detected

CHAPTER FIVE

Discussion

5.1 Quality of white cheese:-

The low fat contents (Table1) of white cheese samples in plastic pack could be due to lipolytic activity of microorganisms on the fat, by the way the findings in this study were not in agreement with Osman (2005) who found the significant increase ($P < 0.05$) in the fat of cheese samples packaged in metal compared to the plastic packages.

Protein content of white cheese sample in paper pack was lower than that in plastic pack this possibly due to the heavy proteolytic action of microorganisms , this result comparable to Bilal (2000) who explained that protein contents of white cheese sample in polyethylene bags were higher than those in anti-acid cans. This result was not agreed with Bayar and Özrenk (2011) who found that protein content of cheese sample in plastic pack was lower than that in skin and cloth packages.

Total solids content of white cheese samples in paper pack was lower than those in plastic pack this might be due to the higher moisture content of cheese .This result was agreed with Abdalla (2007) who studied the total solids content of white cheese in polyethylene bags was higher than that in metallic gallon bags.

The low VFA in white cheese samples in plastic pack could be attributed to the utilization of some of VFA by microorganisms . Our findings were not

in accordance with the study of Hamid (2005) who stated the white cheese samples kept in plastic containers had higher VFA than those in antiacid cans.

The high acidity of white cheese samples in plastic pack was probably due to activity of lactic acid bacteria,.These values were lower than those reported by ElNasri *et al*., (2012) who stated that the acidity of white cheese samples was (1.35 ± 0.60%) for cheese samples in plastic containers. These results were not inline with those obtained by Çakmakçı *et al*. (2011) who represented that cheese samples in plastic package had higher than those in skin pack.

The high ash contents of white cheese samples in plastic pack could be due to the high content of acidity and salt concentration, These values were lower than those reported by ElNasri *et al*., (2012) who found the ash content of white cheese samples was (6.25 ± 2.12%) for cheese samples in plastic containers. This result was disagree with Hamid (2005) and Bilal (2000) who reported that ash contents of white cheese samples in cans was higher than those of cheese kept in polyethylene bags.

The low calcium, Phosphorus and Potassium contents of white cheese samples in paper pack probably attributed to the lipolytic activity of microorganisms on this minerals. This result was not in agreement with Wong *et al.*, (1988) who stated the Phosphorus contents of white cheese samples were not significant differences between cheese samples kept in metal tin and plastic containers.

White soft cheese is widely consumed by the Sudanese population. However, raw milk and cheese are frequently implicated as vehicles of transmission of pathogenic bacteria and with outbreaks reported all over the world (Flowers *et al.,* 1992). Moreover, the pathogenic bacteria in cheeses pose a threat to human health due to the increased number of cases and the severity of symptoms (Heikal *et al.,* 2014). The Total bacterial count gives a quantitative idea of the presence of mesophilic aerobic microorganisms of animal origin. It serves as an important criterion to evaluate the microbial quality of various foods and also the degree of freshness of food (Nanu *et al.*, 2007).

The high TBC in white cheese samples in paper pack could be due to the effect of low acidity of the cheese samples and changes in the environmental conditions during cheese storage which allowed the growth and multiplication of microorganisms. The value in this study was lower than that found by Hamid, (2005) who studied the microbiological compostion of white soft cheese and reported the values of total bacterial count of white cheese samples were (7.52 ± 1.05 Log cfu/ml; 5.48 ± 3.28 Log cfu/ml).These results were not agreed with ElNasri *et al.* (2012) and Ahmed, (1985) who stated that the TBC result indicates significant($P \leq 0.05$) differences between two packaging materials , the total bacterial count of cheese samples kept in plastic containers was lower than those packed in antiacid cans.

Different area (Table 5) was not significantly affected on the total bacterial count of the white cheese. These results were in line with Abdalla and Omer (2017) who found the microorganisms tested were not significantly affected by the different areas in white cheese manufactured under

traditional conditions. The contamination of raw milk with moulds and yeasts is considered to be a reflection of hygienic conditions during the processes of milking and milk storage. The high presence of *yeasts* and *moulds* in white cheese samples in plastic pack could be due to presence of oxygen in the plastic packaged. Our results were in agreement with those reported by Idris and Alhassan (2010) who reported the *molds* and *yeasts* counts of white cheese in different packaging materials were observed the highest values mainly in plastic packages.

Staphylococcus. aureus is capable of producing several enterotoxins that when ingested through contaminated food could cause food poisoning in human with varying intensity (Brightwell *et al.*, 2006) So, presence of *Staph. aureus* in milk and dairy products even in low numbers must be regarded as public health hazard, because it has been established that *Staph. aureus* may lose its viability in food but its enterotoxin still exists. The viability of *Staph. aureus* during the manufacture of dairy products as in cheese depends on the addition of starter culture, salt concentration and storage time (Erkman, 1995). The low count of *S. aurous* in white cheese samples in paper pack could be due to effect of high lactic action of lactic acid bacteria . This result agree with Abdalla *et al.*, (2012) who found the *S. auras* in whit cheese samples kept in plastic lined with polyethylene bags containers.

Coliforms particularly *E. coli* are frequently used in the microbiological analysis of food as an indicator of poor hygienic conditions. Moreover, coliform test is used to measure the quality of practices used to minimize microbial contamination of dairy products. Contamination of cheese with coliforms especially fecal coliforms gives an indication of either direct or

indirect fecal contamination and that considered as a mirror for the degree of disregard of numerous hygienic rules during processing and marketing (Jay, 2000).

The high coliforms in white cheese samples in plastic pack could be due to interaction effect of acidity, similar results observed by Idris and Alhassan (2010) who studied the effect of packaging materials on microbiological properties of Sudanese white cheese found the *coliform* bacteria in cheese samples in plastic packages. Moreover Hamid and El-Owni (2007) detected *coliforms* and *E. coli in* white cheese samples collected in west Darfur.

Escherichia coli was not detected in white cheese sample that attributed to good hygienic practices cheese factory, or due to increase acidity of the cheese. This rseult comparable to Ahmed (2018) who studied the microbiological properties of white soft cheese and found that there was not *E. coli* detected in all the cheese samples.

5.2 Quality of Romi cheese: -

The chemical composition of Romi cheese (Table 8) was significantly affected ($P< 0.01$) by the packing materials. Fat contents of Romi cheese samples in paper pack were higher in comparison with those in plastic pack. The high fat content of Romi cheese samples in paper pack could be due to the low moisture content and high dry matter. This value was similar to Osman *et al.*, (2011), who studies the chemical characteristics of Ras cheese and found the fat content of cheese sample was (29-41%).

Protein contents of Romi cheese were significantly affected (P< 0.01) by the packing materials. The protein content of Romi cheese samples in paper pack was higher than that in plastic pack. The low protein contents of Romi cheese samples in plastic pack may be due to the denaturation of proteins by the heat. These findings were inconsistent with Hayaloğlu *et al.*, (2007) who demonstrated that cheese samples in plastic package had lower protein content than those in skin package.

The low total solids content of Romi cheese samples in plastic pack could be due to the high action of proteolytic and lipolytic microflora on the cheese components. These values comparable to Mijan *et al.*, (2010) who studied the evaluation of quality of Mozzarella cheese found the (Ts %) of Romi cheese sample was (60.3 ± 1.1%).

The low VFA of Romi cheese samples in plastic pack could be attributed to utilization of some of VFA by microorganisms.

The high acidity of Romi cheese samples in plastic pack possibly due to the activity of lactic acid bacteria.This result was in line with Tarakçi and Durmuş (2016) who stated that cheese samples in plastic package was higher than those in skin and coth packages. These values were lower than those found by Osman *et al.*, (2011) who studied the chemical characteristics of of Ras cheese collected from Assiut markets found the acidity % of the cheese sample was (1.15 _1.36).

High ash contents of Romi cheese sample in paper pack could be due to the lower moisture content. These values connected with Mangia *et al.*, (2011) who studied the Physicochemical Properties of Romano cheese found the ash content was (4.15±0.64%).

The low Calcium contents of Romi cheese samples in plastic pack could be due to the degradation of calcium by the lactic acid bactria. Once the low Phosphorous and Potassium contents of Romi cheese samples in paper pack might be due to the lipolytic activity of microorganisms on Phosphorus.

Protein, total solids , titratable acidity, Calcium, Phosphorus and Potassium contents of Romi cheese samples were not significantly affected by the different area. however ash and fat contents of Romi cheese sample were higer in Omdurman samples. However, the volatile fatty acids content of Romi cheese samples was higher in Khartoum north. This variation probably due to effect of storage temperature and the degradation effect of microorganisms of the cheese samples.

The microbial quality and safety of Romi cheese, is the major area of concern for producers, public health authorities and consumers. It depends on the types of microorganisms introduced from raw milk, efficiency of processing and the hygienic practice applied in small or big dairy plant or informal producers. Total counts of bacteria are the most useful indicator for the microbiological status of the cheese. A high viable count often Indicates contamination of raw materials, unsatisfactory sanitation, or unsuitable time and temperature during storage and/or production (Mossel, 1983).

High total bacteria counts of Romi cheese samples in plastic pack could be due to the effect of environmental conditions during storage and low acidity of the cheese samples. The high presence of *yeast* and *molds* in Romi cheese samples in plastic pack could be due to presence of oxygen in the plastic pack. These results were in agreement with Osman *et al.*, (2011) who

studied the chemical characteristics of Ras cheese and yeast and moulds was detected in Ras cheese samples.

Staphylococcus aureus in paper pack was higher than that in plastic pack this could be due to effect of high concentration of lactic acid of lactic acid bacteria, presence of *staphylococcus aurous* in cheese samples indicated to the poor personal hygiene of factory workers, handling and enviromental conditions.

Coliforms and *E. coli* in foods usually indicates a lack of hygiene in handling and production operations, many strains of *E. coli* bacteria are harmless, and may cause serious illness in people (Olsvik *et al*., 2005). Coliforms and *Escherichia coli* were detected in Romi cheese samples in paper pack could be due to the low titratable acidity of cheese samples.

5.3 Quality of Mozzarella cheese:-

Low fat contents of mozzarella cheese samples in plastic pack could be due to lipolytic activity of microorganisms on fat. This value lower than that Mijan *et al*., (2010) who studied the production and effect of storage on the chemical composition of Mozzarella cheese found the fat content of Mozzarella cheese samples was (24.2 ± 0.4%).

The low protein contents of the cheese samples in plastic pack were possibly due to heavy proteolytic action. Moreover these values were higher than those by Sameen *et al*.(2008) who studied the quality evaluation of Mozzarella cheese found the protein content of Mozzarella cheese was (17.77 ± 0, 01%).

The low total solids content of the Mozzarella cheese samples in plastic pack could be due to the action of proteolytic and lipolytic microflora on the cheese components. This value was higher than that found by Abdel Moneim *et al*., (2012) who studied the chemical composition of Mozzarella cheese and reported the total solid content of Mozzarella cheese sample was (50.13 ± 0.003%).

The low content of VFA in Mozzarella cheese samples in plastic pack might be attributed to utilization of some of VFA by microorganisms.

The high acidity of Mozzarella cheese samples in plastic pack in Omdurman could be due to the lactic acid content produced by the activities of bacteria found in the cheese samples which have the ability to ferment lactose. This value higher than that reported by Jooyandeh *et al*., (2016) who studied the physicochemical properties of Mozzarella cheese found the titratable acidity of cheese was (0.35±0.01 %).

High value of ash content of Mozzarella cheese samples in plastic pack could be due to the high content of acidity and salt concentration. This value was higher than that by El Owni and Osman (2009) who stated the evaluation of chemical composition of Mozzarella cheese and found that the ash content of Mozzarella Cheese samples was (2.38 ± 0.41%).

Low Calcium, Phosphorous and Potassium contents of Mozzarella cheese samples in plastic pack may be due to the lipolytic activity of microorganisms on these minerals. however, these values were lower than Abdel- Moneim *et al*., (2012) who stated the chemical composition of Mozzarella cheese found potassium content of cheese was (1.3 ±0.01%).

The different areas (Table 16) was not significantly affected the microbial contents of Mozzarella cheese in plastic pack. This result was in line with Abdalla and Omer (2017) who found the microorganisms tested were not significantly affected by the different areas.

The *Yeast* and *molds* (Table 17) were detected in Mozzarella cheese samples in plastic pack this could be due to presence of oxygen in the plastic pack. *Staphylococcus aureus* was detected in Mozzarella cheese samples in plastic pack which could be due to action of lactic acid bacteria.

Salmonella and *Listeria monocytogens* counts were not detected in all cheese samples. *Salmonella* . *spp* and *Listeria monocytogens* were not detected in white cheese samples. This result comparable with Mojsova et al (2013) who reported that pathogenic bacteria were not detected in cheese sample. This result was agreed with Gamal and Soad (2016) who reported that none of kareish and domiati cheese samples contained detectable level of *Salmonella. spp.* This result was in accordance with Warsama *et al*., (2006) who told that some of *Salmonella .spp* was detected in some samples of Sudanese white cheese. on the other hand this result agree with Amran and Abbas (2011) who reported that pathogenic flora such as *Salmonella* and *Listeria* were detected in some samples of cheese.

CHAPTER SIX

Conclusion and Recommendations

6.1 Conclusion:-

It's concluded that the packing materials showed significant affect (P< 0.01) on the chemical composition of white cheese. The Fat, crude protein, total solids, titratable acidity, ash, Ca, P and K were high in plastic pack, while the (VFA) was low. The packaging materials and the area showed not significant affected on total bacterial count of white cheese. High presence of yeast, mold, *Staphylococcus aureus* and Coliforms were found in white cheese samples in plastic pack. *Escherichia coli* were not detected in white cheese samples. The packaging materials had significant affect (P< 0.01) on the chemical composition of Romi cheese. Fat, crude protein, total solids, VFA, ash and Ca were high in paper pack while titratable acidity, K and P were low. Packaging materials and area had not significant affected on the total bacterial count of Romi cheese. High presence of yeast, mold, were found in Romi cheese samples in plastic pack. However, high presence of Staphylococcus aurous, Coliforms and *E.coli* counts were found in paper pack. Different area were significantly affected (P< 0.01) on the Total solids, volatile fatty acid and titratable acidity of Mozzarella cheese samples, once the fat, Protein , ash , Ca, P and K contents of were not significantly affected by the different area. The different area was not significantly affected on the total bacterial count of Mozzarella cheese. Yeast, molds, and Staphylococcus aureus were found in Mozzarella cheese

samples in plastic pack. Coliforms ,*Escherichia coli* were not detected in mozzarella cheese samples. *Salmonella* and *Listeria monocytogenes* were not detected in all cheese samples.

6.2 Recommendations:-

- From the present study the following recommendations could be written;
- Further work is needed to study the effect of packing materials on vitamins, amino acids and micro elements of cheese.
- To keep the cheeses in good quality the handling and storage conditions should be improved to prevent contamination.
- Further work is required about the processing of Mozzarella and Romi cheese.
- Further work is recommended to study the effect of package materials components on the consumers health.
- To improve the safety of these product efforts to raise awareness of the importance of hygiene barriers and raw milk quality as well as improved process control can be suggested.
- Receiving of raw milk should be carefully monitored and only obtained from suppliers apply good manufacturing practices.
- Strict hygienic measures of cleaning and sanitization of all dairy contact surfaces and hygienic training of plant workers should be applied to avoid contamination.
- Water supply must be clean and comply with the standard requirements, prevention of environmental contamination, good

cleaning and sanitizing of food processing is essential to produce safe and high quality cheese.

- Good conditions of hygiene should be maintained throughout cheese manufacture until consumption to prevent contamination.
- HACCP – based risk assessment, good manufacturing practice and ISO 22000 food safety should be implemented for all stages of manufacture in order to produce safe and good quality dairy product.

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