Chapter one

Introduction:

The problem of preservation of milk samples is common in many countries because the diagnostic laboratories are generally far away from the dairy farming communities; transport of the samples to the laboratory for diagnosis is inadequate (Dunham, 1985). These problems are aggravated by the need for facilities to keep the milk cool in order to minimize bacterial proliferation and sample spoilage prior to examination, as they are generally lacking, Recently scientists have used various milk preservatives (hydrogen peroxide, sodium azide, bronopol, potassium dichromate, boric acid, milkofix, azidiol, ortobor acid) to overcome these problems (Ng- Kwai-Hang, 1982; Hanus *et al*. 1992a; Hanus *et al.* 1992b; Heeschen *et al.* 1994; Saha *et al.* 2003; FOSS Electric 2005). Applying instrumental methods in analyzing raw milk it is allowed to use preservative agents (FOSS Electric 2005). In the literature, it is possible to find various preservatives for each indicator (total bacteria count, fat and protein content, somatic cell count) (Sešķēna, *and Jankevica, 2007).*

Optimization of instrumental methods and precise estimation of milk content and quality indicators, it is necessary to find a preservative that could be used to estimate all of the indicators, mentioned above from one sample vial till the last one. Its common practice here in some areas of Sudan to use lupine seeds into apiece of clean cloth and inserted into the fresh raw milk container during the process of buying their milk as preservative, that why this study is important to know the potentiality of using the extract of lupine as preservative factor on the physicochemical and microbial load of the raw milk.

Objective of the study:

Overall objective:

Is to determine the effect of lupine extract as preservative in cow's milk. **Specific objectives** are:

- To study the physico-chemical properties of raw cow's milk with different levels of lupine extract.
- To determine the microbial load of raw cow's milk with different levels of lupine extract. .
- To determine the shelf life of raw cow's milk with different levels of lupine extract during storage.

Chapter Two

Literature Review:

2.1. Definition of milk:

Milk is the product of the total, full and uninterrupted milking of a dairy female in good health, also nourished and not overworked. It must be collected properly and not contain colostrums (Adib and Bertrand, 2009). Milk is a whitish food generally produced by the mammary secretary cells of females in a process called lactation; it is one of the defining characteristics of mammals. The milk produced by the glands is contained in the udder. Milk secreted in the first days after parturition is called colostrums (Kebchaoui, 2012). The quality of milk is paramount; therefore, it must be properly stored and transported in optimal conditions (Roux *et al*, 1995).

Sudan is the first among the Arab countries and the second in Africa with respect to animal population. According to recent estimates of the livestock, there are about 40 million heads of cattle, 50 million heads of sheep, 43 million heads of goat and 4 million heads of camel (MAR, 2008).Milk production in Sudan is estimated to be about 7.8 million tons per year (MAR, 2007), of which 90% is produced by local breed in traditional sector and 10 % from cross bred by the modern sector (FAO, 2010). The local breeds in Sudan belong to the group of North Sudan Zebu (McDowell, 1972; Sudanimals, 2006). Examples are Butana, Kenana and Baggara; multipurpose breeds that are used for milk and meat production as well as draught power (Payne, and Hodges 1997). The Butana cow is considered to be the best milk producer of the Sudanese zebu breeds (Sudanimals, 2006). The milk production of Sudanese indigenous cattle breeds;

Kenana and Butana *(B.indicus)* were found to be lower than that of Holstein Friesian cattle *(B.taurus)*, even under the same climatic conditions (Ageeb, and Hayes, 2005). Milk is an essential food for human. The majority of milk consumed throughout the world is bovine milk. It is often described as a complete food because it contains all essential nutrients e.g. protein, carbohydrate in the form of lactose, fat, vitamins and minerals (Komorowski and Early, 1992).

2.2. Milk preservation:

2.2.1. Methods of milk preservation

Gould (1996) reported that preservation aims to delay or prevent microbial growth; it must therefore operate through those factors that most effectively influence the growth and survival of microorganisms. He noted that the major preservation techniques employed to prevent or delay spoilage are reduction in temperature, reduction in pH, reduction in water activity and application of heat. Janetschke (1992) reported that the most common preservation methods in the dairy industry include: drying, cooling, freezing, heating irradiation, salting pickling, smoking, preservatives and packaging. FAO/WHO (2005) mentioned that there are several ways in which the spoilage of milk may be controlled, including refrigeration, heat treatment, microfiltration (with or without pasteurization), bactofugation, high-pressure treatment and use of chemical preservatives (including salting at level of 3-12%). Some of these procedures require expensive equipment and are not widely applicable particularly in small – scale dairy production and processing system in developing countries where up to 80% of the milk produced may enter the informal market (Elwell and Barbano, 2006).

2.2.2. Chemical preservation

Hussain and Islam (1990) stated that majority of the dairy farmers in many countries have no ability to install cold room or to buy refrigerator. Similarly heated milk is not generally accepted by the public in the market, another alternative way is to preserve milk with chemical preservatives. They added that recently scientists are using various milk preservatives (H2O2, ethanol, boric acid) to overcome this problem. Lactoferrin and Lysosyme exist in milk and play an anti-microbial role in depriving bacteria from iron and may protect the dry udder from infection (Ghibaudi *et al*, 2000).

Abd Elwahab (1993) suggested that to improve hygienic quality and to lengthen the shelf life of milk, some efforts have to be put on milk treatment like refrigeration, heat treatment and chemical preservation. She added that refrigeration and heat treatment are rather expensive to rely on in Sudan, thus leaving the chemical preservation as a possible alternative to adopt, Yuan (2001). Found that protein and peptide such as lactoperoxidase, lactoferrin, bacteriocins, Lysosome and xanthine oxidation, occurring naturally in milk and have antimicrobial properties, FAO/WHO (2005) strongly discourages the preservation of milk by chemical means, except the application of H2O2 at native LPS and in the case of H2O2; it must be completely destroyed before consumption (Ozer *et al.*, 2003).

2-2.3.The Chemical composition of milk:

2.2.3.1. Changes in milk composition:

The composition of milk may change due to differences in relative rates of synthesis and secretion of milk components by the mammary gland. Variations are due to differences among species, between individuals within a strain, and between conditions affecting an individual, Conditions affecting the cows may

include the weather or seasons and the stage of lactation (Kilic 1994; Haenlein, 2003).

2.2.3.1.1. Breed:

The US mostly uses milk from cows of the larger breeds, such as Holsteins and Brown Swiss' because of the lower fat content and greater milk production. Breeds such as the Guernseys and the Jerseys have higher fat contents in their milks. Both the Guernseys and the Jerseys have a fat content of 5.2%, where as the Holsteins and the Brown Swiss' have fat contents of 3.5%. (Kilic 1994; Haenlein, 2003)

2.2.3.1.2. Diet:

The composition of the cows' diet and the form in which they are fed affect the composition of milk and especially milk fat. High fat and/or low roughage diets can reduce the fat content of milk. Diet has small effects on protein content and none on lactose content. The seasonal effect is due to the changes in the diet throughout the year. (Kilic 1994; Haenlein, 2003).

2.2.3.1.3. Stage of lactation:

When mammals give birth, their first secreted milk is called colostrum, and it differs greatly in composition from regular milk. Colostrums contain more mineral salts and protein and less lactose than normal milk. Also, fat content, calcium, sodium, magnesium, phosphorus, and chloride are higher in colostrums than in normal milk. Whey content is about 11% in colostrums as opposed to 0.65% in normal milk. Colostrums contain extremely high immunoglobulin (Lg) content. Igs accumulate in the mammary gland before parturition and transfer immunity to the baby cow. This immunoglobulin protects the baby cow until it can establish its own Immunity, The variation in milks and milk yield within a

species depends on so many factors. Some of these factors are genetics, stage of lactation, daily variation, and parity, type of diet, age, udder health and season (Kilic 1994; Haenlein, 2003).

The process ability and quality of milk products such as cheese, butter are influenced significantly by these factors (Lind mark – Mansson *et al*, 2000). District, climatic conditions and lactation periods are known as seasonal changes which have influences on the milk composition. Especially, there is a negative correlation between environmental temperature and the amount of milk fat and protein. When temperature is increased the solid fat tends to decrease. Ng-Kwai-Hang *et al*. (1982) and Lacroix *et al*. (1996) have reported that percentage of fat, protein, casein and all the fraction of nitrogen have been influenced by the seasonal variations. The light-to-dark ratio can also induce marked changes in milk yield and composition (Casati *et al.*, 1998).

2.2.3.2. Milk compositions:

2.2.3.2.1. Milk fat:

The MFG is formed in the secretary cells of the mammary gland. Precursors of milk lipid globules are formed at the endoplasmic reticulum and are transported through the cytosol as small droplets of triglycerides covered by a non-bilayer of polar phospholipids and proteins. During transport the droplets grow in size, apparently due to droplet-droplet fusion (Dylewski *et al*. 1984; Deeney *et al*.1985). At the apical plasma membrane, the droplets are secreted from the epithelial cell. During secretion, the droplets are covered by the plasma membrane and finally pinched off into the lumen of alveolus. The precursors of milk lipid globules have a group of polypeptides on the surfacein common with the membrane of the endoplasmic reticulum (Deeney *et al*., 1985). However, it is still unknown where in the endoplasmic reticulum network the lipid droplets are

formed (Mather and Keenan, 1998). Another unknown mechanism is how the lipid droplets are transported to the apical plasma membrane of the cell.

Milk fat is excreted in the form of small droplets, which, in cow's milk, range from 1 to 12 in diameter with the mean of about 3 Triacylglycerols are the predominant lipids in bovine milk, accounting for 97-98% of total lipid. The remaining lipids are diacylglycerols, monoacylglycerols, phospholipids, free fatty acids, and cholesterol and its esters (Muir, 1992).Furthermore, there are two different theories of how the fat droplets are secreted. One theory is that the lipid droplets reach the apical region of the cell, where they are secreted and covered by cellular membranes. The lipid droplets are gradually coated with plasma membrane until a narrow neck of membrane and cytoplasm remains. At the point when the membrane in the neck fuses together, the fat globule is secreted and expelled into the alveolar lumen (Mather and Keenan, 1998).

Likewise casein should be covered by a secretory vesicle and the content of such may then be released from the apical surface by exocytosis. The hormones prolactin and oxytocin affect the release of the lipid globules and is thought to affect the final size of the MFGM (Ollivier- Bousquet, 2002).Triglycerides are the major fraction of neutral lipids in the MFGM. However, most of this is believed to originate from contamination (from the core of the MFGM) during isolation of the membrane (Walstra 1974 and 1985). Whole milk contains 308 to 606 mg cholesterol /100 g fat (Jensen, 2002 and Walstra *et al*. 1999).

Reported the cholesterol content in the MFGM to be 0,2 mg/m2. However, the proportion of cholesterol decreases through lactation (Bitman & Wood, 1990). Mono- and triglycerides, FFA and glycospringolipids are also present in the MFGM. The latter of the four consists of neutral glycolipids and gangliosides. The quantity of gangliosides is about 8µg/mg membrane protein and the

composition is identical to apical plasma membranes of the secretory cells in the mammary gland (Jensen, 2002).

Phospholipids form the basic bilayer in biological membranes in which the non polar tails are arranged side-by-side and turn towards the lipids. The polar head groups are orientated towards the aqueous environment. A suggestion of the proposed structure of the MFGM. There is a layer of high melting triglycerides surrounding the core fat. Xanthine oxidase is assumed to be a peripheral membrane protein since it does not containing a long sequence of 15 non polar amino acids to function as membrane anchor (Mather and Keenan, 1998). However, xanthine oxidase is probably associated with the inner membrane (Mather and Keenan, 1998).

2.2.3.2.1.1. *Composition of the milk fat globule:*

Many studies and reviews have dealt with the composition of fatty acids in milk (Bitman and Wood, 1990; Jensen, *et al*. 1991; Bitman *et al*. 1995and Jensen, 2002). The composition of fatty acids in milk is affected by feed and breed. The fatty acids containing from 4 to 14 carbon atoms are synthesized from the acetate and β-hydroxy butrate which are products of the fermentation of carbohydrates in the rumen. This pathway is called *de novo* synthesis. Some of the palmitic acid (C16:0) is also synthesized *de novo*. Long chain fatty acids, *i.e.* those containing 16 or more carbon atoms, are provided to the glands from the blood stream and originate directly from the diet or from the adipose tissue. Palmitic (C16:0) and stearic (C18:0) acids passed through the rumen unchanged while unsaturated fatty acids are subjected to biohydrogenation by the reducing environment caused by the microorganisms in the rumen, resulting mainly in stearic acid together with a smaller amount of oleic acid (C18:1) (Borsting *et al*. 2003) Furthermore, stearic acid derived from the diet is partly converted to oleic acid by stearoyl-CoA desaturase, in the intestines and the mammary tissue.

Unsaturated lipid supplements are often protected/encapsulated to avoid biohydrogenation in the rumen. Moreover, high amounts of unsaturated lipids in the rumen result in incomplete biohydrogenation, so some of the linoleic acid (C18:2) and linolenic acid (C18:3) is transformed into conjugated linoleic acids (CLA). Specific isomers 12 of CLA together with *trans*-C18:1 in the rumen has a negative effect on the *de novo* fat synthesis resulting in lower fat content of the milk (Bessa *et al*. 2000).

2.2.3.2.1.2. Lipolysis in milk:

Lipoprotein lipase (LPL) is the enzyme mainly responsible for lipolysis in raw milk. It originates from the mammary gland, where it is involved in the uptake of blood lipids for milk synthesis. The enzyme is active in lipid-water interfaces. Its optimum temperature is 33°C, and pH optimum is about 8.5. It is a relatively heat labile enzyme which is mostly inactivated by a high temperature-short time heat treatment. In milk, LPL is mainly associated with the casein micells (Hohe *et al.*, 1985). LPL is brought into contact with the triglycerides when the MFGM is disrupted and casein coats the formed lipid-water interface. The enzyme is activated by apo-lipoprotein CII from the blood which assists LPL to bind onto the fat globule (Bengtsson and Olivecrona, 1982). In spite of the high amount of LPL in milk, lipolysis is limited since milk fat is protected by the membrane and raw milk is normally stored at temperatures far below the optimum temperature of LPL. Furthermore, the products of the hydrolyses of the triglycerides, the FFA, inhibit the enzyme presumably due to that the FFA binding to the LPL. Furthermore, the proteose-peptone component 3 is found to inhibit LPL (Cartier, *et al*. 1990; Girardet et al. 1993).

2.2.3.2.2. Milk Protein:

The proteins in milk fall into two distinct types, caseins (82.2%) and whey proteins (17.8%) that can be isolated by using various separation technologies (Huffman and Harper, 1999). Milk protein has a very high nutritional value, due not only to its high essential amino acid content, but also to its high digestibility.The proteins in milk are of great quality, that is to say, they contain all the essential amino acids, and elements that our bodies cannot produce. It is important to remember that proteins are the building blocks of all living tissue. Milk proteins have roughly the same composition as the egg protein, except for the amounts of methionine and cystine, significantly lower. Indeed, the sulfur amino acids are the limiting factors in milk. Casein and, even more, the complex milk protein contains good proportion of all amino acids essential for growth and maintenance (Konte, 1999). The denomination crude protein (CP) includes protein and non-protein nitrogen (including urea). The protein content is an important feature of the milk. The TP determines the Average composition and distribution of milk proteins (FAO, 1998).

2.2.3.2.2.1. Casein

The four major caseins that exist naturally in milk are αs1 caseins; αs2, *B* and *k*. Caseins are distinguished by their low solubility at pH 4.6 and are differentiated on the basis of the distribution of exchange and sensitivity to precipitation by calcium, (Brule et al., 1997). Among the most studied casein is casein k (k-CN), probably because of its importance in the stability of the micelle and its role in dairy processing. The k-CN is also the only casein having carbohydrate residues in its constitution (Fox and Mulvihill, 1992). Caseins (α, β and κ) in the presence of Calcium phosphate, form stable casein micelles (colloidal phase), which are balanced with the soluble phase of milk (St.

Gelais et al., 1992). It is possible to adjust the balance in terms of temperature, pH and the addition of salts. So long as the lactic acid bacteria convert lactose into lactic acid, it lowers the pH of the milk thereby decalcifying the casein micelles. There is another way to destabilize casein micelles by using an enzyme such as chymosine.

2.2.3.2.2.2 Whey protein

Other milk proteins are present in the whey serum and whey proteins are defined as soluble proteins in the whey after precipitation of caseins at pH 4.6 and at 20°C (De Wit, 1981). Serum proteins include a first protein fraction (80%) consisted of β-lactoglobulin (β-LG), α-lactalbumin (α -LA Da), bovine serum albumin (BSA) and immunoglobulin. A second non-protein fraction (20%) is composed of proteose, peptone and nitrogen compounds (Filion, 2006).

2.2.3.2.3. Milk Lactose:

Lactose is a disaccharide comprised of D-glucose linked to D-galactose. The sugar in raw milk may exist in two different crystalline forms, and, which differ in their properties. Lactose is a useful source of dietary energy and is thought by some workers to promote the absorption of calcium from the diet (Muir, 1992). Both types of lactose are widely used in the manufacture of pharmaceuticals. In the production of capsules or tablets it may be employed as a diluents, bulking agent, filler, or excipient, and in powders as a bulking agent. Characteristics such as particle size make different grades of lactose suitable for different applications (Martindale, 1996). Although lactose is a sugar, it does not have a sweet flavor. Its concentration varies slightly in milk (4.5 to 5.2 g / 100 g) contrary to the concentration of fat that of lactose cannot be easily modified by feeding and true step of a dairy race to another. It is used as substrate during the fermentation of milk by lactic acid bacteria, differing in the fermented products

such as yoghurt and cheese. It plays a role in fermented milk production. The amount of lactic acid produced by lactic acid bacteria in a fermented milk product depends not only on the bacterium itself (the bacterial strain more less active) and operating parameters, but also on the available amount of lactose bacteria. The buffer milk power also plays an important role as we shall see later (Fillion, 2006).

2.2.3.2.4. Milk Ash:

Mineral elements occur in milk and dairy products as inorganic ions and salts, as well as part of organic molecules, such as proteins, fats, carbohydrates and nucleic acids. The chemical form of mineral elements is important because it determines their absorption in the intestine and their biological utilization. The mineral composition of milk is not constant because it depends on lactation phase, nutritional status of the animal, and environmental and genetic factors.

All essential mineral elements can be found in milk because by definition it contains the nutrients required for growth of the young (Bates and Prentice, 1996). Milk and dairy products are an important source of dietary minerals in many European countries, accounting for 10-20 % of daily dietary intake. However, the content of major and trace elements in milk depends upon the content of these elements in soil and cattle feed, which varies considerably among and within countries (Dobrzański *et. al*., 2005; Malbe *et al*., 2010). Also, the thermal treatment of milk may have influence on mineral composition in the way that concentration of dietary minerals in consumer milk is lower than concentration in raw milk, with the exception of iron, which is higher in consumer milk (Mable *et al*., 2010).

2.2.3.2.5. Vitamins:

Levels of vitamin A, D and E are variable; depending on the season as there is a slight increase during the pasture season (spring-summer). They are fat-soluble, so it is found in fat and can be lost during skimming. Other vitamins are water soluble and are found in the serum. In the case of ascorbic acid (C), it is present in small quantities in fresh milk and is destroyed by contact with air and also during pasteurization (Schrdos, 1982). For cow milk, the milk processing techniques can significantly change the amount of vitamin C (Florence, 2010).

2.2.3.2.6. Enzymes:

Enzymes are specific globular proteins produced by living cells. Each enzyme has its isoelectric point and is susceptible to various denaturing agents such as pH change temperature, ionic strength, organic solvent (Carole and Vignola, 2002)**.**

2.2.5. The nutritional value of milk:

The nutritional value of milk is particularly high due to the balance of the nutrients that compose it. The composition varies among animal species and breeds within the same species, and also from one dairy to the other, depending on the period of lactation and diet. For instance, goat milk is 88% water and 11.4% solids; it contains 3.2% fat and 8.13% of fat solids. It is also comprised of calcium (0.11%), phosphate (0.08%) and magnesium (0.21%). In general, goat milk compared to cow milk is less rich in lactose, fat and proteins, but has similar mineral content. Milk contains several groups of nutrients. Organic substances are present in about equal quantity and are divided into elements builders, proteins, and energy components, carbohydrates and lipids. It also comprises functional elements, such as traces of vitamins, enzymes and

dissolved gases, and contains dissolved salts, especially in the form of phosphates, nitrates and chlorides of calcium, magnesium, potassium and sodium. It also contains dissolved gases (5% by volume), mainly carbondioxide (CO2), nitrogen (N) and oxygen (O2) (Gautheron and Lepouze, 2012).

Milk is a complex mixture of fats, proteins, carbohydrates, minerals, vitamins and other minor constituents dispersed or dissolved in water (Harding, 1999)**.** Milk is an important part of the human diet and its nutritional significance is apparent from the fact that daily consumption of a quart (1.14 liters) of milk furnishes approximately all the daily requirements from fat, calcium, phosphorus, riboflavin, one half of the protein, one third of vitamin A, ascorbic acid, thiamine and one fourth of calories needed daily by an average individual **(**Bilal and Ahmad, 2004**)**

2.2.6. The lupines and their native:

Lupines' (lupinus) are native to the Mediterranean region, Eastern Africa and North and South America. Crop lupines' can generally survive in poor winter environments such as deep sandy, infertile soil and poorer climates that the warm season soybean is not adapted to (Smart *et al*, 1988).

Lupines have been established in Western Australia successfully since the 1970's and three crop species of lupines-sweet narrow-leafed lupine (*L. angustifolis*), white lupine (*L. albus*) and yellow lupine (*L. luteus*) are currently cultivated. *Lupinus mutabilis* is currently under development in Western Australia due to its high protein and oil contents (Clements *et.al*. 2008).It is known as lupines in the United States, as turmus in the Middle East and Tawari in Latin America. The plant is characterized by having various flowering spikes in large range of colors (Kurzbaum *et al*., 2008). Lupines' are mostly utilized by stock feed manufacturers in compound feed rations. There is increasing utilization in aquacu`lture (Glencross *et al*.,(2003)|;Glencross, 2005).

2.2.6.1. Centers of origin

Four different centers of origin have been proposed for the genus lupines. These include the Mediterranean region (including Northern Africa), North America, South America, and East Asia. Today, approximately 90 % of the recognized species are found in alpine, temperate and subtropical zones of North and South America, which ranges from Alaska to Southern Argentina and Chile. The remaining species are native to the Mediterranean region and Africa. But due to their larger seeds, most of the economically important species come from the Mediterranean region, (ARC, 2009).The lupines potential health benefits, Due to low glycemic index of their seeds, it was found that lupine kernel fibers have appetite suppression (Archer *et al*. 2004). And cholesterol lowering properties (Hall *et al*. 2005), that they lower blood glucose and insulin levels (Hall *et al*. 2005).

2.2.6.2. Chemical composition of lupine:

Chemical composition differences are related to implicit differences due to location and season or climatic conditions, with slightly variation among varieties (Wolko *et al*., 2011). But in general, chemical composition related to ash, fiber and ether extract contents are close to the reported range for other *Lupinus* species (Hill, 1977; Yanez *et al*., 1983; Zdunczyk *et al*., 1994; Ruiz and Sotelo, 2001).

Legumes represent, together with cereals, the main plant source of proteins in human diet. They are also rich in dietary fibre and carbohydrates (Rochfort and Panozzo, 2007). Minor compounds of legumes are lipids, polyphenols, and bioactive peptides (Pastor-Cavada *et al*., 2009). Lupine is a good source of nutrients, not only proteins but also lipids, dietary fibre, minerals, and vitamins (Martínez-Villaluenga *et al*., 2009). There is virtually no starch (2 %) in any of

the lupine species. This is in marked contrast to crops such as field peas and chickpeas, which can have 50-70 % of the cotyledon weight as starch and have low protein and oil content, and the soybean with 15-20 % oil and high protein content. Their crude protein content ranges from about 28 to 42 %. There are variations in the protein content between species and cultivars as a result of the characteristics of the growing conditions and soil types (Martínez-Villaluenga *et al.*, 2006a).

Crud protein

Legumes play an important role in human nutrition since they are rich sources of protein, calories, certain minerals and vitamins. In African diets legumes are also, the major contributors of protein and calories for economic and cultural reasons (El Maki *et al*., 2007).

Amino acids content

Legume proteins are rich in lysine and deficient in sulphur containing amino acids, whereas cereal proteins are deficient in lysine, but have adequate amounts of sulphur amino acids (Eggum and Beame, 1983).

Crud fibre:

The dietary fibre is composed of total dietary fibre (TDF), which includes both soluble (SDF) and insoluble dietary fibre (IDF). In terms of health benefits, both kinds of fibres complement with each other. A well balanced proportion is considered when there is 70-50 % insoluble and 30-50 % soluble DF (Grigelmo-Miguel *et al*., 1999).

Crud fat:

The fat level in lupine is ranked third after ground nut (*Arachis hypogeae L.*) and soybean (*Glycin max*) among legumes (Uzun *et al*., 2007). The lipid contents of *L. albus* are similar to other species of the genus lupinus like *L. campestris* (Jimenez-Martinez *et al*., 2003).The oil extracted from *L. albus* seed consist various types of fatty acids. The fatty acids of the oil from the raw seed are composed of more of unsaturated fatty acid and small percentage of saturated fatty acids. This means *L. albus* can be a potential source of considerable amount of useful vegetable fat. Among the unsaturated fatty acids, majority oleic and linolenic acids are found (Uzun *et al*., 2007). The high content of ω-6 and ω-3 fatty acids, make the crop a healthy alternative edible oil source (Joray *et al*., 2007).

2.3. Microbial of the milk:

2.3.1. Hygiene production of milk

Milk is a magnificent medium for growth of microorganisms and therefore a risk of quick microbiological deterioration of quality is present from time of milking to the time of use (IDF, 1994). The general standard of hygiene applied for milk production in developing countries is poor and hand milking is almost a common practice in developing countries (Chye *et al*.,2004).Hygienic control in raw milk are that the milk should be obtained from healthy animals and from animals not been treated with antibiotics or other veterinary drugs, which can be transferred to milk (Murphy and Boor, 2000).Also it includes prevention of contamination of milk by stable environment and milking equipment as well as controlling temperature and time in order to minimize the growth of pathogens (IDF,1994 AND Murphy and Boor,2000).

2.3.2. Sources of raw milk contamination

Milk is synthesized in specialized cells of the mammary gland and is virtually sterile when secreted in to the alveoli of the udder (Tolle, 1980). Due to its high nutritional value, milk represents a good medium for bacteria and other microorganisms. The main sources of contamination in the farm are cow's udder and body, utensil, milking machines, stable and the transportation equipment (Hunderson, 1971).Generally, contamination of raw milk occurs from three main sources: within the udder, the exterior of the udder, and from the skin of the handlers and the surface of storage equipments(Bramley, and Mckinnon,1990).

2.3.2.1. Microbial contamination from within the udder

The teat cistern, teat canal and teat apex may be a Colonized by variety of microorganisms, microbial contamination from Within the udder of health animals is not considered to contribute significantly to the total numbers of microorganisms in the bulk tank or during refrigerated storage (Murphy and Boor, 2000), they also stated that a cow with mastitis has the potential to shed large numbers of microorganisms in to the milk supply.

2.3.2.2. Microbial contamination from the exterior of the udder

The exterior of the cow's udder and teats can contribute to contamination of raw milk by microorganisms .These microorganisms are either naturally associated with the skin of animals or the environment in which the cow is housed and milked (Brito *et al*, 2000). The teat skin is one of the main sources of the microbial contamination of raw milk as well as a source of mastitis infection (Brito *et al*., 2000).

2.3.2.3 The handling and storage equipments

Cleaning of milking system influences the total bacteria count in milk at least as much as any other factor, milk residues left on equipment contact surfaces supports the growth of variety of microorganisms. Organisms are

considered to be natural inhabitants of the teat canal apex, and skin generally does not grow significantly on soiled milk contact surfaces or during refrigerated storage of milk. In general, environmental contaminations (i.e., from bedding, manure, feed …etc) are more likely to grow on soiled equipment surfaces than are organisms associated with mastitis (Olson *et al*., 1980).

2.3.3. Type of bacteria found in milk

Milk is a complex fluid containing a mixture of carbohydrates, protein, fat, and minerals in different physio-chemical status and forms. Its comprehensive nutritional properties and high moisture content make it an excellent medium for supporting microbial growth (FAO, 1997). Milk provides a favorable environment for the growth of microorganisms (O, Connor, 1995).Microbes can enter milk via the cow, air, feeds, milk handling equipment and milker. Bacteria types commonly associated with milk.

2.3.3.1. Lactic Acid Bacteria (L A B)

Frazier (1995) reported that the L A B are a group of bacteria able to ferment lactose of milk to Lactic acid and also used as starter culture in the production of cultured dairy products such as yogurt. Examples of these microorganisms are:

(1)Streptococci: *Streptococcus lactic* and *Streptococcus cremoris*.

(ii)Lactobacilli: *Lactobacillus casei, Lactobacillus lactis and Lactobacillus bulgaicus.*

(iii)Leuconostoc: *Leuconostoc mesenteroides*.

2.3.3.2. Coliforms

These are indicator organisms associated with the presence of pathogens and can cause rapid spoilage of milk (Frazier, 1995). He also mentioned that they are killed by HTST treatment, their presence after treatment is indicative of contamination

2.3.3.3. Spoilage microorganisms

The most common spoilage microorganisms in milk and dairy products are Gram negative rod –shaped bacteria, Gram positive spore forming bacteria, lactic acid producing bacteria and yeast and moulds (IDF, 1994). The defects that can occur in milk due to microbial growth are off flavor, lipolysis with development of rancidity, gas production, souring due to fermentation, coagulation of milk protein, viscous or ropy texture and discoloration (Banwart, 1981). Lactic acid producing microorganisms (*Streptococcus spp*, *Lactobacillus spp*. And *Leuconostoc spp*) spoil milk by fermenting lactose to produce acid (IDF, 1994).

2.3.3.4. Pathogenic microorganisms

Milk borne human infection and intoxication is due to *campylobacter spp*, *Listeria monocytogenes* ,*Salmonella spp* ,*Staphylococcus spp* , *yersinia entercolitica* ,*Escherichia Coli* ,*Bacillus cereus* ,*Clostridium perfringes* ,*Clostridium botulinum and Streptococcus zooepidemicus* (IDF,1994). Giovannini (1998) reported that various zoonotic agents can be transmitted to human through milk . In Germany, Deutz et,al. (1999) examined 133 raw cow's bulk milk from 3 dairies for the presence of Camplyabacter jejuni, C.Lari, E.Coli O157, Listeria moncytogenes and Salmonella. However, they found no Salmonella spp. was found but Camplyobacter spp, L.moncytogenes and EC.Oli O157 were found.

2.3.4. Bacteriological aspect of raw milk.

2.3.4.1. Total bacterial cell count

The examination of foods of the presence, types and numbers of microorganisms and /or their products is basic to food microbiology. Some methods of analysis are better than others; every method has certain inherent limitation associated with its use. The most widely used test as a general indication of good hygienic milk production is the standard plate count.

Harding , (1999) report shows that total bacterial count of raw milk from individual producers should not exceed $1X10^4$ cfu/ml and that for bulk milk should not exceed $3X10^4$ cfu/ml , while for pasteurized milk the bacterial load should not exceed $2X10^3$cfu/ml (FDA,2001). The mean counts Per ml for TBC,Psychrotrophic and thermophilic were 12×10^6, $7.5\times10^3$and 9.1×10^3, respectively and TBC less than 10^6 cfu/ml is used as basic standard by MCC in the price incentive program (Chye *et al* .,2004).

2.3.4.2. Coliform bacteria

They are groups of bacteria including the genera Escherichia, Citrobacter, Enterobacter and Klebsiella (Al. Ashmawy, 1990). Hussein (2001) found that the coli form count of raw milk was high in Khartoum North (log 10 3.071 ± 0.689 cfu /ml) followed by Khartoum (log 10 3.071 ±0.749 cfu /ml) and Omdurman (log 10 3.051 ±1.01 cfu /ml).

2.3.4.3. *Escherichia coli*

It's a member of the family Enterobacteriaceae, Gram negative non spore forming straight rod bacteria (Rea and Fleming, 1994) .They mentioned four pathogenic categories of *E.coli* which include enterophogenic (EPEC). enterotoxigenic (ETEC), entroinvasive (EIEC)and entrohaemoragenic (EHEC). Padhye and Dayle (1992) stated that *E.coli* was recognized as an important human pathogen, and illness caused by *E.coli* infection ranged from self watery diarrhea to life threatening manifestations such as heamolytic uraemic syndrome. Dasilva, *et al*. (2001) isolated enterophathogenic E.coli (EPEC) from pasteurized milk which may represent a potential risk for children.

2.3.4.4. Pseudomonas aeroginosa

Pseudomonas aeroginosa is not a common cause of mastitis, but has been observed to be of major concern in some herds. (Schalm *et al* 1971). in Egypt, Khalil, (1992) found that Pseudomonas aeroginosa isolated from raw milk was resistant to penicillin, ampicilin, erythromycin streptomycin and susceptible to polymixin.

2.3.5 .Grading of raw milk

Raw milk under topical condition was graded according to many factors which include the number of microorganisms present in milk, oder, flavor, amount of sediment,appearance and temperature (Chandan *et al* .; 1979).They also reported that milk was graded as good when it had total bacterial count (TBC)of 5.0 $\times 10^5$ cfu/ml or less , satisfactory when the (TBC)ranged between 5.0×10^5 to 5.0 $\times 10^6$ cfu /ml and bad when the (TBC) was more than 5.0×10^6 cfu/ml. According to the US Department of Health Education and Welfare (1953), milk was graded as grade A when the bacterial count was less than 2.0×10^4 cfu/ml, grade B when the bacterial count ranged between 2.0×10^4 to 1.0 $\times 10^6$ cfu/ml and grade C when the bacterial count was more than 1.0 $\times 10^6$ cfu/ml.

Chapter Three:

Material and Methods:

The present study was conducted during 2016 at the laboratories of College of Animal Production Sciences and Technology, Sudan University of Sciences and Technology).

3.1. Materials:

3.1.1. Source of milk:

Six liters (6) of fresh caw's milk were purchase from the College of Animal Production Sciences and Technology, Sudan University of Sciences and Technology Dairy farm at Kuku area.

3.1.2. Source of lupine:

Lupine seeds were brought from Kuku market at Khartoum state.

3.2. Methods:

3.2.1. Lupine extracts preparation:

The lupine seeds grinded in to fine powder (flour), before added to hundred mls of distilled water for different lupine powder weights, they sterilized at 55 ºC for twenty four hours (24) hrs, after that 0.5%, 1%, 1.5% and 2% by weight were soaked into distilled water and kept at the refrigerator temperature for 24 hrs, then filtered by filter papers (size 42). .

3.2.2. Treatments:-

In this study five treatments were carried out .First sample is a control, in which raw fresh cow milk left at room temperature without lupine extract, in the second, third, fourth and fifth treatments (0.5%, 1%, 1.5%, and 2%) levels of lupine extract were added to the raw cow's milk (four hundred mill of milk for each sample) samples respectively. The raw milk samples in all treatments left for 0, 1, 2, 3, 4 and 5 hours at room temperature. The physicochemical analysis of the milk samples were carried out for (protein, fat, titratable acidity, total solids not fat, PH and ash) at each specified time. Each treatment repeated in duplicated.

3.2.3 Chemical analysis of milk:

The chemical composition of milk and treatment samples (protein, fat, total solids not fat and PH) was determined by **Lactoskan (made in BULGARIA, SUPLY 12-14V DC50W)** (fresh milk analyzer), while titratable acidity, and ash were determined by AOAC (2009) methods.

3.2.3.1. Ash content:

The ash content was determined according to AOAC (2009). 10 mls of milk were weighted in to suitable clean dry crucible and evaporated to dryness on steam bath, and the crucibles were placed in muffle fume at 550 ^{c0} for 1.5 – 2 hrs, cooled in dissector and weighted . The ash content was calculated as follows:

Ash % = w1/w0X100
Where =

w1 == weight of ash

W0 == weight of sample.

3.2.3.2. Titratable acidity:

Titratable acidity was determined according to AOAC (2009).Ten mills of milk samples were placed in to a clean porcelain dish and three to five drops of phenolphthalein indicator was added, the sample was titrated against 0.1NaoH till a faint color lasted for at least 30 seconds, then the titratable acidity of each sample calculated as follows:

Titaratable acidity = T/W

Where:

T = Titration figures
W = Weight of samples.

3.3. Methods[2]

3.3.1 Microbial analysis (total bacteria count):

The plate agar medium was used for the determination of the total bacteria count according to Ramakant (2006).

3.3.1.1 The preparation of Nutrient agar (the medium):

The manufacturer's instructions were followed by dissolving 28 grams of powder of plate agar medium in a liter of distilled water, heated to boiling point and sterilized in an autoclave at 121 ºC for fifteen minutes.

3.3.1.2 Culturing:

Serial dilusions were made for each sample then from each dilution, fifty micro mili liter (mml) was transferred in to sterile Petri dishes (duplicate) followed by addition of melted, cooled (45-46 ºC) plate count agar, mixed thoroughly by

rotating the first in one direction and then in the opposite direction . When the medium has solidified, the dishes were incubated in an inverted position 37 ºC for 24 hours.

3.3.1.3 Counting:

The number of colony-forming unites (cfu) in each dilution was obtained by multiplying the number of colonies in reciprocal of each dilution.

3.4. Statistical analysis:

Statistical analysis was done using, Statistical Package for Social Science (SPSS, version 16. 2007). General linear models were used to estimate the effect of different levels of lupine extract, storage periods and the interaction between them on the chemical composition, microbial load of the raw cow's milk. Least significance different (LSD) was used for separation between the treatments mean. The level of significance (0.05) was used in this study.

Chapter Four

Results

4.1. Effect of different levels of lupine extract on chemical composition of fresh cow's milk.

Results in table (1) illustrated the effect of different levels of lupine extract on physicochemical characteristics of cow milk. The results indicated that there is significant difference ($p<0.05$) in the protein content within the treatments.

The data showed that the highest protein content (3.24±0.22 %) was in the control milk sample, while the lowest one (3.02±0.17%) was recorded in the cow's milk treated with 0.5% lupine extract.

Fat content of the fresh milk sample was significantly ($p<0.05$) higher (4.04±0.76%), while the lowest value (3.35±0.21%) was found in the milk treated with 1% lupine extract (table 1).

The results in table (1) indicated that Total Solids Not Fat was significantly different ($p<0.05$) within the treatments. The higher TSNF content (8.78±0.64%) was in the control milk sample, however the lower fat value (8.27±0.46%) was found in the milk treated using 0.5% lupine extract.

pH content was significantly ($p<0.05$) affected by the different levels of lupine extract within all treatments. The lowest pH level (6.73±0.11) was scored by the milk with 0.5% lupine extract, while the highest one (7.01±0.36) was scored by the milk treated with zero lupine extract (table 1).

Acidity of the cow milk samples was not significantly ($p<0.05$) affected by the treatments. Highest acidity percent (0.22±0.01) was for the control, 1.5%,

and 2% lupine extract, while the lowest value (0.21±0.02%) was for 0.5% and 1% lupine extract (Table 1).

The ash data showed in the table (1) which was not significantly ($p<0.05$) affected by the concentration of lupine extract. The highest ash content (0.67±0.08%) was found for 0.5% and 1.5% of lupine extract; however the lowest value (0.63±0.19%) was recorded in control milk samples.

4-2- Effect of storage period on physicochemical characteristics of fresh cow's milk.

Data in table 2 shows the effect of storage time on the physicochemical characteristics of the fresh cow's milk.

The result indicated that the storage time had significant ($p<0.05$) effect on the acidity content of the fresh cow's milk, (table 2). The highest acidity content (0.23±0.01%) was at fourth hours and the lowest value (0.21±0.01%) was at the control. The data indicated that the storage time had significantly ($p<0.05$) affected the fat content. The highest fat content (3.84±1.07%) was found at zero time. The lowest one ((3.23±0.26%) reported at fifth hour (Table 2).

The study demonstrated that (table 2), the storage time had significantly (($p<0.05$) affected the T.S.N.F content. The highest T.S.N.F content (8.59±0.33) was recorded at second hour, while the lowest one (8.26±0.26) was found at the fifth hour.

Table1: Effect of different levels of lupine extract on the physicochemical composition of fresh cow's milk.

Treatment	Chemical composition					
	Protein%	**Fat%**	**T.S.N.F**	**pH**	**Acidity**	**Ash%**
Control	3.24±0.22^{a}	4.04±0.76^{a}	8.78±0.64^{a}	6.73±0.11^{c}	0.22±0.01	0.63±0.19
0.5%	3.02±0.17^{c}	3.64±1.08^{b}	8.27±0.46^{b}	7.01±0.36^{a}	0.21±0.02	0.67±0.08
1%	3.02±0.10^{bc}	3.35±0.21^{c}	8.29±0.26^{b}	6.83±0.12^{b}	0.21±0.01	0.66±0.11
1.5%	3.13±0.15^{b}	3.41±0.26^{bc}	8.53±0.40^{ab}	6.82±0.09^{bc}	0.22±0.01	0.67±0.08
2%	3.08±0.10^{bc}	3.45±0.29^{bc}	8.51±0.31^{ab}	6.79±0.07^{bc}	0.22±0.02	0.66±0.09
Sig	**	**	*	**	N.S	N.S

Mean values bearing different superscripts within columns are significantly different ($p^{<0.}$05). L.S = levels of significances.

* NS = not significance.

Table 2: Effect of storage period on physicochemical characteristic of fresh cow milk.

Storage time	Chemical composition					
	Protein%	**Fat%**	**T.S.N.F**	**pH**	**Acidity**	**Ash %**
zero-time	3.11±0.29^{ac}	3.84±1.07^{a}	8.54±0.77	6.78±0.05^{bc}	0.21±0.01^{b}	0.69±0.10^{a}
1 hr	3.06±0.13^{ac}	3.47±0.34^{bc}	8.38±0.35	6.96±0.36^{a}	0.21±0.00^{b}	0.67±0.09a^{b}
2 hrs	3.15±0.12^{ab}	3.69±0.75^{ab}	8.59±0.33	6.88±0.07^{ab}	0.210.01^{b}	0.60±0.18a^{b}
3 hrs	3.13±0.19^{a}	3.64±0.66^{ab}	8.50±0.54	6.98±0.17^{a}	0.21±0.01^{b}	0.58±0.06^{b}
4 hrs	3.00±0.12^{c}	3.61±0.46^{ab}	8.26±0.35	6.76±0.08^{ac}	0.23±0.01^{a}	0.70±0.09^{a}
5 hrs	3.14±0.08^{a}	3.23±0.26^{c}	8.58±0.23	6.67±0.06^{c}	0.23±0.02^{a}	0.69±0.07^{a}
Sig	NS	**	NS	***	***	NS

Mean values bearing different superscripts within columns are significantly different ($p^{<0.}$05).

L.S = levels of significances. * NS = not significance.

Statistical analysis revealed that storage time had no significant (p˂0.05) effect on ash content, total solids not fat and protein content of the fresh cow milk, samples (table 2).

4.3. Effect of different levels of lupine extracts and storage time on physicochemical characteristics of fresh caw's milk.

Protein content of fresh milk was not significantly ($p<0.05$) affected by the levels of lupine extract and storage time. The lowest protein content (2.85±0.30 %) was observed at zero time in the milk treaded with 0.5% lupine extract, while the highest one (3.56±0.25 %) was reported at zero time in the control milk sample (Table 3).

Results in table (4) showed that fat content was significantly ($p<0.05$) affected by the levels of lupine extract and storage time. The lowest fat content (2.99±0.09%) was recorded at fifth hour in the 0.5% of milk samples. And the highest one (5.80±0.04 %) was found at zero time in the milk sample with 0.5% lupine extract.

Total solids not fat content of the milk samples were not significantly ($p<0.05$) affected by the levels of lupine extract and storage time. The highest T.N.F content (9.73±0.69 %) was for the control milk at zero time. While the lowest one (7.86±0.74%) was for milk with 0.5%lupine extract, (table 5).

Data in table (6) shows the pH content of the milk samples which was significantly ($p<0.05$) affected by the different levels of lupine extract and storage time. The lowest pH (6.57±0.02) was reported at fifth hour in the milk with zero lupine extract, while the highest one (7.53±0.60) was for one hour in the milk with 0.5% lupine extract.

The acidity content of the milk samples was not significantly ($p<0.05$) affected by the different levels of lupine extract and storage time. the lowest acidity (0.20±0.00%) were recorded at zero time in the milk made with 0.5% and

1% lupine extract, while the highest one (0.25±0.04%) was found for fifth hour in the milk made of 2% lupine extract table (7).

Results in table (8) show the ash content of the milk samples which was not significantly ($p<0.05$) affected by the different levels of lupine extract and storage time. The lowest ash content (0.41±0.42) was reported at second hour in the milk made with zero lupine extract, while the highest one (0.8±0.00) was found at zero hour and fourth hour in the milk made of zero lupine extract and 1% lupine extract.

Table 3- Effect of different levels of lupine extracts and storage time on the protein of fresh milk.

Storage Time	**Lupine concentration**				
	Control	0.5%	1 %	1.5%	2 %
Zero. time	3.56±0.25	2.85±0.30	2.97±0.01	3.02±0.04	3.17±0.11
1 hr	3.26±0.07	2.99±0.04	2.99±0.05	3.11±0.11	2.96±0.03

2 hrs	3.10±0.29	3.25±0.07	3.14±0.08	3.17±0.04	3.12±0.01
3 hrs	3.27±0.04	2.90±0.07	3.17±0.04	3.32±0.25	3.01±0.04
4 hrs	3.11±0.27	3.02±0.09	2.89±0.00	2.99±0.04	3.01±0.01
5 hrs	3.15±0.12	3.13±0.00	3.03±0.01	3.19±0.05	3.20±0.09
Sig	*				

Mean values bearing different superscripts within rows are significantly different ($p^{<0.}$05).

* L.S = levels of significances. * NS = not significance.

Table4: interaction between different levels of lupine extract and storage time on fat content (%) of fresh cow milk.

storage time	Lupine concentration				
	Control	0.5%	1 %	1.5%	2 %
Zero. time	3.20±0.69	5.80±0.04	3.13±0.04	3.49±0.11	3.59±0.13
1 hr	3.56±0.33	3.03±0.05	3.28±0.07	3.59±0.16	3.87±0.24
2 hrs	5.04±0.54	3.21±0.32	3.47±0.07	3.26±0.06	3.51±0.06

3 hrs	4.54±0.76	3.23±0.93	3.23±0.11	3.79±0.20	3.44±0.01
4 hrs	4.27±0.40	3.63±0.49	3.71±0.01	3.20±0.02	3.23±0.11
5 hrs	3.66±0.04	2.99±0.09	3.30±0.13	3.13±0.01	3.06±0.09
Sig	***				

Mean values bearing different superscripts within rows and columns are significantly different ($p^{<0.}$05).

L.S = levels of significances. * NS = not significance.

Table 5: interaction between different levels of lupine extract and storage time on total solid not fat content (%) of fresh cow's milk.

Storage time	**Lupine concentration**				
	Control	**0.5%**	**1 %**	**1.5%**	**2 %**
Zero. time	9.73±0.69	7.86±0.74	8.10±0.01	8.35±0.25	8.67±0.28
1 hr	8.93±0.18	8.20±0.08	8.17±0.19	8.52±0.32	8.10±0.09
2 hrs	8.49±0.79	8.90±0.21	8.57±0.21	8.50±0.14	8.50±0.00
3 hrs	8.39±0.47	7.93±0.21	8.65±0.09	8.94±0.91	8.60±0.62

4 hrs	8.52±0.73	8.18±0.38	8.01±0.13	8.18±0.10	8.45±0.19
5 hrs	8.61±0.32	8.55±0.00	8.28±0.01	8.72±0.13	8.74±0.25
Sig	NS				

Mean values bearing different superscripts within rows are significantly different ($p^{<0.}$05).

L.S = levels of significances. * NS = not significance.

Table 6: Effect of different levels of lupine extract and storage time on pH of fresh cow's milk.

Storage time	**Lupine concentration**				
	control	**0.5%**	**1 %**	**1.5%**	**2 %**
Zero. time	6.78±0.04	6.79±0.04	6.72±0.11	6.81±0.02	6.79±0.02
1 hr	6.79±0.05	7.53±0.60	6.83±0.0	6.85±0.01	6.79±0.01
2 hrs	6.81±0.01	6.97±0.06	6.93±0.01	6.87±0.04	6.83±0.01
3 hrs	6.81±0.04	7.27±0.13	7.00±0.01	6.94±0.05	6.91±0.2
4 hrs	6.63±0.09	6.83±0.04	6.83±0.02	6.78±0.01	6.73±0.03
5 hrs	6.57±0.02	6.68±0.04	6.71±.02	6.67±0.02	6.71±0.00

Sig	**

Mean values bearing different superscripts within rows are significantly different ($p^{<0.}$05).

L.S = levels of significances. * NS = not significance.

Table 7: Effect of different levels of lupine extract and storage time on titratable acidity content (%) of fresh cow's milk.

Storage time	**Lupine concentration**				
	control	**0.5%**	**1 %**	**1.5%**	**2 %**
Zero. time	0.23±0.01	0.20±0.00	0.20±0.00	0.22±0.01	0.22±0.00
1 hr	0.22±0.00	0.21±0.01	0.21±0.00	0.22±0.01	0.21±0.01
2 hrs	0.22±0.01	0.21±0.01	0.21±0.00	0.22±0.02	0.21±0.00
3 hrs	0.22±0.00	0.21±0.01	0.21±0.01	0.21±0.01	0.22±0.01
4 hrs	0.24±0.02	0.22±0.01	0.23±0.01	0.24±0.01	0.22±0.01
5 hrs	0.22±0.03	0.24±0.01	0.23±0.04	0.23±0.01	0.25±0.04
Sig	NS				

Mean values bearing different superscripts within rows are significantly different ($p^{<0.}$05).

L.S = levels of significances. NS = not significance.

Table 8: Effect of different levels of lupine extract and storage time on Ash content (%) of fresh cow's milk.

storage time	Lupine concentration				
	control	**0.5%**	**1 %**	**1.5%**	**2 %**
Zero. time	0.80±0.00	0.70±0.14	0.60±0.14	0.70±0.00	0.65±0.07
1 hr	0.70±0.07	0.70±0.00	0.70±0.00	0.65±0.07	0.65±0.21
2 hrs	0.41±0.42	0.65±0.07	0.65±0.07	0.65±0.07	0.65±0.07
3 hrs	0.55±0.07	0.65±0.07	0.55±0.07	0.55±0.07	0.60±0.00
4 hrs	0.65±0.07	0.60±0.00	0.80±0.00	0.75±0.07	0.70±0.14
5 hrs	0.60±0.00	0.70±0.14	0.70±0.07	0.70±0.00	0.70±0.00
Sig	NS				

Mean values bearing different superscripts within rows are significantly different ($p^{<0.}$05).

L.S = levels of significances. * NS = not significance.

4.4. Effect of different levels of lupine extract on Total Bacteria Count of fresh cow's milk.

The data in table (9) showed that significant ($p < 0.05$) variations were found in the TBC of the different treatments. The highest total bacteria count (6.59±0.17log cfu/gm) was found in the control milk samples, while the lowest one (6.46±0.12log cfu/gm) was recorded for milk sample with 1.5% of lupine extract.

4-5- Effect of storage time on total bacteria count of raw cow's milk.

The results in table (10) explained that the total bacteria count was significantly ($p < 0.05$log cfu/gm) affected by the storage time. The highest total bacteria count (6.58±0.16 log cfu/gm) was reported at fourth hour, while the lowest one (6.44±0.23 log cfu/gm) was recorded at first hour.

4.6. Effect of different levels of lupine extracts and storage time on microbiological characteristics of fresh cow's milk:

Results in (table 11) indicated that total bacteria count was not significantly ($p^{<0.}$ 05log cfu/gm) affected by the levels of lupine extract and storage period. The lowest total bacteria count (6.34±0.24 log cfu/gm) was recorded at one hour at 1%, while the highest one (6.71±0.07 log cfu/gm) was at fourth hour in the milk sample contained zero lupine extract (control).

Table 9: Effect of different levels of lupine extract on Total Bacteria Count of fresh cow's milk.

Parameter	**Lupine concentrations**					
	control	**.5%**	**1%**	**1.5%**	**2%**	**sig**
T.B.C	6.59 ± 0.17^{a}	$6.58\pm0.18a^{a}$	6.51 ± 0.17^{ba}	6.46 ± 0.12^{b}	6.48 ± 0.14^{b}	**

Mean values bearing different superscripts within rows are significantly differently ($p^{<0.}$05). L.S = levels of significances.

NS = not significance.

Table10: Effect of storage Time on Total Bacteria Count of raw cow's milk.

Bacterial count	**Storage time**						
	Zero hr	1hr	2hrs	3hrs	4hrs	5hrs	sig

T.B.C	6.53±0.13^{ab}	6.44±0.23^{b}	6.52±0.15^{ab}	6.52±0.13^{ab}	6.58±0.16^{a}	6.57±0.14^{a}	*

Mean values bearing different superscripts within rows are significantly different ($p^{<0.}$05).

* L.S = levels of significances. * NS = not significance.

Table11: Effect of different levels of lupine extract and storage time on Total Bacteria Count of fresh caw's milk.

Storage time	Lupine concentration				
	control	0.5%	1%	1.5%	2%
Zero-time	6.52±0.12	6.63±0.06	6.55±0.10	6.43±0.09	6.49±0.20
1 hr	6.57±0.16	6.42±0.32	6.34±0.24	6.44±0.19	6.45±0.26
2 hrs	6.57±0.14	6.62±0.09	6.44±0.20	6.42±0.16	6.54±0.05
3 hrs	6.51±0.29	6.48±0.11	6.48±0.02	6.55±0.08	6.55±0.07
4 hrs	6.71±0.07	6.75±0.04	6.59±0.09	6.43±0.11	6.41±0.13
5 hrs	6.69±0.12	6.56±0.13	6.67±0.10	6.50±0.04	6.44±0.11
Sig	NS				

Mean values bearing different superscripts within rows are significantly different ($p^{<0.}$05).

L.S = levels of significances. NS = not significance.

Chapter Five

Discussion

5.1. Effect of different levels of lupine extract on the physicochemical characteristics of fresh cow milk:

The protein of the untreated milk samples had highest value in comparison with others treatments (table 1), This could be attributed to high moisture content in the milk samples from different levels of lupine extract which may decrease the total solids of milk, these results are in agreement with, those reported by Wolko *et al*., (2011) who stated that the lupines extracts deactivate substances such as the lectins and protease inhibitors that reduce protein digestion and availability. However no variations were observed in the protein contents of the milk samples with lupine extract.

Fat content of the milk samples increased with the increasing levels of lupine extract (table 1), this could be due to the high amount of fat in lupines, and these results coincided with those of, Kroger (1971).

The total solids not fat of the milk samples decreased with the increasing levels of lupine extract (table 1), This could be due to the proteolytic'activities of lupine extract and these results were not in accordance with those of Gupta, (2010), who studied the compositional change in cross bred and local cow milk as affected by 0.3 and 0.5% formalin preservative. No significant difference was recorded in lactose, total solids, fat and specific gravity on addition of formalin in milk.

The pH of the milk samples showed high values with increasing levels of lupine extract, this could be due to the breakdown of protein 'activities of lupine extract, these results were not in line with those studied by, Giolitti, et al (1949), who found that no changes for lactose, fat, total nitrogen and pH after the addition of 0.04% by weight of H2O2 to milk.

The acidity and ash contents of the milk samples were not affected by lupine extract addition (table 1), These results were in contrast with, those reported by Sandhu et al. **(**1984).

5.2. Effect of storage period on the physicochemical characteristics of fresh cow milk.

The protein, total solids not fat and ash content in this study were not affected significantly by the storage period (table 2). These results, were not in agreement with those reported by ISO (1999), who studied that the effect of **C6H7KO2** (**potassium sorbate**) on protein content measurement was the reverse, i.e.

protein content in tested samples stored at 4 °C and 20 °C increased by 0.20 % and 0.39 %, correspondingly.

The fat content (table 2) was higher at zero time then decreased at fifth hour which is the lowest one; these probably due to the lipolytic activities, these results were in lines with those of (Seskena, and Janevica, *2007*).

The PH (table 2) of the sample with lupine extract showed high values at first, second and third hour than zero time, these might be due to antimicrobial included in the lupines extract inhibited the lactic acid bacteria, these results are in lines with those of Baltess (1998).

The acidity of raw milk samples did not show increase till fourth and fifth hours (table 2), the increasing in the acidity might be due to the breakdown of lactose in to lactic acid by the lactic acid bacteria, and these results are similar with those reported by Minzner and Kroger (1974).

5.3. Interaction between different levels of lupine extract and storage period on the physicochemical characteristics of fresh cow milk:

In Table (3), the results of protein values decreased with the levels of lupine extract and also with the storage period, these probably due to the breakdown of protein by the microorganisms in the raw milk sample with lupine extract, these results agreed with those of Yuan (2001).

Results in table (4) showed that fat content was significantly ($p < 0.05$) affected by the levels of lupine extract and storage period, the lowest fat content (2.99±0.09%) was recorded at fifth hour in the milk without lupine extract, this might be due to the hydrolysis of fats, the results agreed with those of Kroger (1985).

Total solids not fat content (Table 5) of the milk samples were not significantly ($p<0.05$) affected by the levels of lupine extract and storage time, the highest T.N.F content (9.73±0.69%) was for the control milk at zero time, the decrease in T.S.N.F values with an increased in the levels of lupine extract and time, these might be due to the moisture content in the extract, these results agreed with those *of* Boghra and Borkhatriya, (2003).

The lowest pH (6.57±0.02) was reported at fifth hour in the milk without lupine extract, these results mean the PH values increased with the levels of lupine extract and decreased with increasing the time, and this might be due to the preservative effect of the lupine extract, these results are in accordance with those of Baltess (1998), who stated that the important factors influence the efficiency of preservatives include an initial microbial count in the product, microbial species, temperature and pH of environment.

The acidity content (table 7), of the milk samples was not significantly ($p<0.05$) affected by the different levels of lupine extract and storage time, this might be due to the low activity of antimicrobial factors in lupine extract, these results were not in line of those of Dawood et al, (1974), who found that addition of 0.1% formalin to milk increased the titratable acidity from 0.175 to 0.190%.

Results in table (8) showed that the ash content of the milk samples was not significantly ($p<0.05$) affected by the different levels of lupine extract and storage time.

5.4. Effect of different levels of lupine extract on total bacteria count of fresh cow's milk.

The data in table (9) showed that lupine extract significantly ($p<0.05$) affected total bacterial count, the effective concentration of lupine extract is 1.5 up to 2%, and the lowest total bacterial count of milk samples with 1.5 and 2% lupine

extract could be due to inhibition effect of lupine extract on the total bacterial count, these results confirmed those of (Sesken and Janevica, *2007*).

5.5. Effect of storage period on total bacteria count of fresh cow's milk.

The results in table (10) explained that the total bacteria count was significantly ($p^{<0}$.05) affected by the storage time. The highest total bacteria count (6.58±0.16 log cfu/gm) was reported at fourth hour, this agreed with those of Erdemoglu et al. (2009).

5.6. Interaction between different levels of lupine extract and storage period on total bacteria count of fresh cow's milk.

Results in (table11) indicated that total bacteria count was not significantly ($p^{<0.}$ 05) affected by the levels of lupine extract and storage, these might be due to the weak antibacterial effect of lupine extract, these results not in line with those reported by Al-Kerwi, et al (2005), who studied the antibacterial effect of milk proteins which mediated by the reaction of hydrogen peroxide (H2O2), that is thought to be a major antibacterial substance. Lactoperoxidase, a known milk peroxidase, when combined together with H2O2 and iodide, produce a potent anti-bacterial system known as the Lactoperoxidase system.

Chapter Six

Conclusion and Recommendations

6.1. Conclusion

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

- The quality of raw cow's milk was improved with the addition of the lupines extract.
- The lupines extract significantly ($p < 0.05$) affected the protein, fat, total solids not fat and PH contents of raw milk samples.
- The storage period significantly ($p < 0.05$) affected the fat, PH and acidity and while no significant effect on protein, total solids not fat and ash contents of raw milk samples were observed.
- The microbiological characteristics of raw milk (total bacteria count) was significantly ($p < 0.05$) affected by the increase levels of lupines.

- The storage period significantly ($p < 0.05$) affected the microbiological characteristics of the fresh milk.

6.2. Recommendations:

The following recommendations were made.

- Further studies will be required for the effective agent in the lupine extract as preservative for raw milk.
- The use of lupine extract in the preservation of raw milk under refrigeration conditions required further studied.
- Intensive research should be done on the nutritional values, vitamins, minerals, enzymes of the raw milk samples preserved by lupine extract.

REFERENCES:

Abd Elwahab, Wafa. M. (1993). Use of hydrogen peroxide as a dairy Preservation in milk destined for cheese making (white soft cheese). M.Sc., Thesis. University of Khartoum. Sudan.

Adib, A., Bertrand, S. (2009). Risk Analysis transfer pesticides to milk. Institute of Livestock. National Interprofessional Centre: 9.

Ageeb, A. G. and Hayes, J. F. (2005). Genetic and environmental effects on the productivity of Holstein-Friesian cattle to the climatic conditions of central Sudan. Trop. Anim. Health Prod. 32, 33-49.

Al-Kerwi, A.A.E., AL-Hashimi, H.A. and Salman, M.A. (2005). *Asia Pacific Journal of Clinical Nutrition*,; 14(4).

Al-Ashmawy, A. M. (1990).Handbook of Food Hygiene: Fluid milk, dairy products, fat, oils and eggs, EL Fardoos –Publishing Co, Cairo, Egypt.

AOAC (2009). Association of Official Analytical Chemists.Food chemistry June 2009, vol.114 (3): 1141-1146, doi: 10.1016/…

Archer, B.J. (2004). Effect of fat replacement by inulin or lupine-kernel fiber on sausage patty acceptability, postmeal perceptions of satiety and food intake in men. British Journal Nutrition 91: 591-599.

Baltess V. (1998). *Food Chemistry*. Fourth Edition. University of Latvia, Riga. 478 p. (in Latvian).

Bates, C.J., Prentice, A. (1996): Vitamins, minerals and essencial trace elements. *Drugs and Human Lactation* 533-607.

Banwart, G. I . (1981**)**.Basic Food Microbiology. Second edition, Avipublishing Co, New York, USA, pp112 - 135.

Bengtsson, G. and Olivecrona, T. (1982). Activation of lipoprotein lipase by apolipoprotein CII: Demonstration of an effect of the activator on the binding of the enzyme to milk-fat globules. *FEBS Letters 147*, 183-187.

Bessa, R. J. B., Santos-Silva, J., Ribeiro, J. M. R. and Portugal, A. V. (2000). Reticulo-rumen biohydrogenation and the enrichment of ruminant edible products with linoleic acid conjugated isomers. *Livestock Production Science 63*, 201-211.

Bilal, M.Q. and Ahmad A. (2004). Dairy Hygiene and Disease Prevention. Pakistan Vet. J, 25.

Bitman, J. and Wood, D. L. (1990). Changes in milk fat phospholipids during lactation. *Journal of Dairy Science 73*, 1208-1216.

Bitman, J., Wood, D. L., Miller, R. H., Wilk, J. C. and Moore, E. D. (1995). Comparison of lipid composition of milk from half-Danish jersey cows and United States jersey cows. Journal of dairy science 78,655-658.

Boghra, V.R.and Borkhatriya, V.N. *(*2003**).**Physico-chemical properties and compositional profile of milk samples as affected by formalin preservation- A review. Indian J. Dairy Sci, *56* (2), 65-71.

Børsting C.F., Hermansen J.E. and Weisbjerg M. R. (2003). *Fedtforsyningens betydning for mælkeproduktionen*. In; Kvægets ernæring og physiology, bind 2 Fodring og produktion. DJF rapport, husdyrbrug nr. 54. (Eds. F. Strudsholm og 34 K. Sejersen). Danish Institute of Agricultural Sciences, Foulum, Denmark. pp. 133-152.

Bramley, A. J .and Mckinnon, C.H. (1990).The Microbiology of raw milk.In: Dairy Microbiology .R. K. Robinson (ed),Vol.(1).Elsevier APPL . Sci., New York. p. 163- 208.

Brito, J .R.F.; Paiva – e – Brito –M .A**.**V**.;** Verneque – R - da – Sciencia – Rural (2000).30, 5:847- 850.

Brulé, G., Lenoir, J. and Reneuf, F. (1997). Les micelles de Caséine et la coagulation du lait. Dans le fromage: de la science à d'assurance qualité. ECRA et gillis J-C (Ed), lavoisier TES. DOC, Paris: 89.

Carole L, V. (2002). Science technologie du lait. Edit. Fondation de technologie laitière du Québec Inc., Canada: 599.

Cartier, P. Chilliard, Y. and Paquet, D. (1990). Inhibiting and activating effects of skim milks and proteose-peptone fractions on spontaneous lipolysis and purified Lipoprotein-lipase activity in bovine-Milk. *Journal of Dairy Science 73*, 1173- 1177.

Casati, M.R., Cappa, V., Calamari,L. Calegari F. and Folli, G. (1998). Effects of the season on milk yield and on some milk characteristics in cows. Scienzae Tecnica Lattiero-casearia, 49: 7-25.y

Chandan, R . C . and Hedrick, T. I .(1979).Farm sanitation and production of good milk quality. Indian Dairy man, 31: 793 – 798. Countries. IDF Special issue, 9002,pp 88 – 89.Belgium.

Chye, F. Y.; Abdullah, A. and Ayob, M.K.(2004).Bacteriological quality and safety of raw milk in Malaysia. Food Microbiology.21:535 - 541.

Clements, J.C., Sweetingham, M.W., Smith, L., Francis,G., Thomas, G. and Sipsas, S. (2008). Crop improvement in Lupines mutabilis for Australian Agriculture – progress and prospects. IN: J. Palta and J. Berger (Eds.) Proceedings of the 12th International Lupine Conference, Fremantle, Western Australia.

Dawood, A.E.; Naghmoush, M.R.; Nofel, A.A. (1974**)**. The effect of certain additives on acidity and formal number of milk. Ala. J. Agric. Res., *22*, 73-77. Cited in *Dairy Sci. Abstr.* 38:8238.

Dasilva, Z. N. D. A.; Cunha, A.S.; Lins, M . C.; Carneiro, L. D.E.A.M,; Almeida A.C., and Queuro, M. L.(2001).Isolation and serological identification of enteropathogenic *Escherichia coli* in pasteurized milk in Brazil ,Rev. Sande publica ,35 (4):375-379.

Deutz,A.; Pless, P. and Kofer, J.(1999).Examination of raw cow and ewe milk for human pathogens. Ernahrung, 23(9):359 - 362.

Deeney, J. T., Valivullah, H. M., Dapper, C. H., Dylewski, D. P. & Keenan, T. W. (1985). Micro lipid droplets in milk secreting mammary epithelial cells: evidence that they originate from endoplasmic reticulum and are precursors of milk lipid globules. *European Journal of Cell Biology 38*, 16-26.

DeWit, J.N. (1981). Structure and junctional behavior of whey proteins. Netherlands Milk and dairy journal. 35 : 47 – 54.

Dobrzański, Z., Kołacz, R., Górecka, H., Chojnacka, K. and Bartkowiak, A. (2005). The Content of Microelements and Trace Elements in Raw Milk from Cows in the Silesian Region. *Polish Journal of Environmental Studies 14*, 685-689.

Dunham, J.R. and M. Kroger. 1985. Milk preservatives. Dairy herd improvement.AvailableSource:http://www.inform.umd.edu/EdRes/Topic/AgrEnv/ndd/dairy/MILK_PRESERVATIVES.

Dylewski, D. P., Dapper, C. H., Valivullah, H. M., Deeney, J. T. and Keenan, T. W. (1984). Morphological and biochemical characterization of possible intracellular precursors of milk lipid globules. *European Journal of Cell Biology 35*, 99-111.

Eggum, B. O., and Beame, R. M. (1983).The nutritive value of seed proteins. *In* "Seed protein biochemistry, genetics and nutritive value" (W. G. P. H. Muller, ed.), pp. 499–531. The Hague, Junk.

Elwell, M.W. and Barbano, D.M. (2006). Use of microfiltration to Improve fluid milk quality. J. Dairy Sci., 89 (E. Suppl.): E10- E30.

El Maki, H. B., AbdelRahaman, S. M., Idris, W. H., Hassan, A. B., Babiker, E. E., and El Tinay, A. H. (2007). Content of antinutritional factors and HCl-extractability of minerals from white bean (Phaseolus vulgaris) cultivars: Influence of soaking and/or cooking. *Food Chemistry* **100**, 362-368.

Erdemoglu, N. Ozkan, S. Duran A *et al* (2009). Analysis and antimicrobial activity of alkaloid extract from *Genista vuralii*. Pharm Biol 47: 81-85.

FAO (1998). Organisation des Nations Unies pour l'alimentation et l'agriculture, Le lait et les produits laitiers dans la nutrition humaine, Collection FAO: Alimentation et nutrition. P 28,

FAO, (2010). FAOSTAT database (online).Available: www.faostat.fao.org

FAO/WHO (2005). Benefits and potential risks of the lactoperoxidase System of raw milk preservation. Technical meeting. FAO, Headquarters 28 November - 2 December, (2005). Rome, Italy.

FAO (1997).Report on Application of membrance and Separation technology to food processing in developing countries. Proceeding of the Expert Consultation Held in FAO, Rome. From 21 – 24 October(1996).

FDA (2001**).**Grad A pasteurized milk Ordinance. Center for Food Safety & Applied Nutrition. Section 1-7. Cited in http://www.cfsan.fda.gov/ear/pmo01-2.html.

Frazier W. C., West HOFFD. C. Food Microbiology, 4 th Ed Reprint (1995**).**Tata McGraw Hill Publishing Co Ltd. New Delhi.

Fillion. M.M. (2006). Amélioration de la stabilité thermique du lait par modulation du potentiel d'oxydoréduction. Thèse: pp.23 – 447.

Florence, C.L. (2010). Qualité nutritionnelle du lait de vache et deses acides gras, voies d'amélioration par l'alimentation. Ecole nationale vétérinaire d'ALFOR. Thèse. Doctorat vétérinaire. P 51.

FOSS Electric. (2005). *CombiFossTM 6000FC Operator's Manual.* FOSS Electric A/S. 110 p.

Fox, P.F., Mulvihill, D. (1992). Milk protein: molecular colloidal and functional proprieties. Journal of Dairy research. 49: 679 – 693.

Gautheron, M. and Lepouze, A. (2012). Le lait, UN aliment indispensable.

Ghibaudi, E. M.; Laurenti, E.; Beltramo P. and Ferrari, R.P. (2000). "Can estrogenic radicals, generated by lactoperoxidase, be involved in the molecular mechanism of breast carcinogenesis?". Redox Rep., 5 (4): 229–235.

Giolitti,G.AttiSoc.Ital.Sci.vet.**1949**,*3*,543.http://whqlibdoc.who.int/monograph/WHO_MONO_48_(p423).

Girardet, J. M., Linden, G., Loye, S., Courthaudon, J. L. and Lorient, D. (1993). Study of mechanism of lipolysis inhibition by bovine milk proteose-peptone component. *Journal of Dairy Science 76*, 2156-2163.

Glencross, B., Hawkins, W. and Curnow.J. (2003). Evaluation of the variability in chemical composition and digestibility of different lupine (Lupines angustifolius) kernel meals when fed to rainbow trout (Oncorhynchus mykiss).Aquaculture Nutrition 9: 305-315.

Glencross, B.D.(2005). Seeding a Future for Grains in Aquaculture Feeds Part III. Proceedings of a Workshop, 14 April (2005) Fremantle (WA). p. 92.

Gould, G.W. (1996). Methods of preservation and extension of shelf life. Int. J. Food Micro. 33: 51-64.

Grigelmo-Miguel, N., Abadias-Seros, M. I., and Martin-Belloso, O. (1999). Characterisation of low-fat high-dietary fibre frankfurters. *Meat Science* **52**, 247-256.

Gupta, H.C.L.; Gupta, D. (2010), Compositional change in cross bred and local cow milk as affected by formalin preservative. Pantnagar J. Res. *8* (2), 219-221.

Haenlein, G., (2003). Nutritional value of dairy products of Ewe and goat milk. RetrievedJanuary28.http://ag.udel.edu/extension/information/goatmgt/gm-10.htm.

Hall, R.S., Johnson,S.K., Baxter A.L. and Ball. M.J (2005). Lupine kernel fibre-enriched foods beneficially modify serum lipids in men. European Journal of Clinical Nutrition 59: 325-33.

Hanus O., Gencurova V., Gabriel B., Zvackova I. (1992a). Comparison of the effectiveness of Milkofi x, a preservative preparation, with traditional preservative agents in the determination of somatic Cell count in milk samples using afluoro-optic-electronic method. *Vet. Med.* 37: 91–99.

Hanus O, Gencurova V. and Zvackova I. (1992b). Testing Milkofi x, a new preservative preparation for Milk samples used for infrared analysis of milk components. II. Verification of its preservative Effects in relation to infrared analysis. *Vet. Med.* 37: 33–43.

Harding, F. (1999)**.** Milk Quality. Chapman and Hall Food Science Book, Aspen Publishers, Inc. Gaithersburg, Maryland, Aspan. **Harding F. (1999**).Milk Quality. A chapman and Hall Food Science Book. Aspen publishers Inc. Gaithersburg, Maryland. First edition Pp.44 - 59.

Heeschen W. H., Ubben E. H. and Rathjen G. (1994). Somatic Cell Counting in Milk: the Use of the Principle of Flow Cytometry for Somatic Cell Counting (Somacount) and Comparison with the Results Obtained with the Fluorescent Optical Principle (Fossomatic 360*)*. Bentley Instruments, INC, Minnesota. P, 33.

Hill, G.D.(1977). The composition and nutritive values of. Lupine seed. Nutr. Abs. Rev.,47:511-519 .http:// whqlibdoc. who.int/monograph /WHO _MONO 48_(p423).pdf.

Hohe, K. A., Dimick, P. S. & Kilara, A. (1985). Milk lipoprotein lipase distribution in the major fractions of bovine milk.. *Journal of Dairy Science 68*, 1067-1073.

Huffman, L.M. and Harper, J.W. (1999). Symposium: Marketing dairy value through technology. Maximizing the value of milk through separation technologies. J. Dairy Sci., 82: 2238-2244.

Hussain, M. S. and Islam, M. N. (1990). Studies on the preservation of milk with hydrogen peroxide. Bangladesh J. Animal Sci., 18: 75-80.

Hunderson, J. L. (1971).The fluid milk industry, 3 rd edition. The Avi publishing Company. Pennsylvania, USA.

Hussain, H. A. (2001).Microbiological and antibiotic profile of milk in Khartoum State. M.Sc. University of Khartoum.

IDF (1994).Recommendations for the hygienic manufacture of milk and milk based products. International Dairy federation, No 292. Belgium.

ISO (E). (1999). *Whole Milk – Determination of Milkfat, Protein and Lactose Content–Guidance on the Operation of Mid-infrared Instruments.* Th e International Organization for Standardization, Geneva. 27 p.

Janetschke, P. (1992). Methods of preserving foods. J. of Dairy Sci., 8: 51-54.

Jensen, R. G. (2002). The Composition of bovine milk lipids, Invited Review. *Journal of Dairy Science 85*, 295-350.

Jensen, R. G., Ferris, A. M. and Lammi-Keefe, C. J. (1991). The composition of milk Fat. *Journal of Dairy Science 74*, 3228-3243.

Jimenez-Martinez, C., Hernandez-Sanchez, H., and Davila-Ortiz, G. (2003). "Lupines: An Alternative for De-bittering and Utilization in Foods," CRC press, LLC, Spain.

Joray, M. L., Rayas-Duarte, P., Mohamed, A., and Van Santen, E. (2007). Coated Lupin Bean Snacks. *Journal of Food Quality* **30**, 267-279.

Khalil , N. G.(1992).Occurrence,detection and significance of pseudomonas aeroginose in raw milk. Assiut. Vet. Med. J. 28. (55). 152-157.

Kebchaoui J (2012). Le lait composition ET propriétés. Co operations universitaire (2012 -2013) entre la faculté polydiscplinaire de Taroudant (MAROC) l'enil de Besancon mamirolle région Franche compte (France). ENIL. Mamirolle (25620): 1 – 4.

Kilic, A. and S. Kilic, (1994). Feeding and milk. Bilgehan Press. Izmir.

Komorowski, E.S. and Early, R. (1992). Liquid milk and cream. in Early, R. (ed) The Technology of Dairy Products. VCH Publishers, Inc., New York, p. 1.

Konte, M. (1999). Le lait ET les produits laitiers. Développement de systèmes de productions intensives en Afrique de l'ouest. Université de Nouakchott (R.I.M) Faculté des Sciences ET Technologies des aliments, B. P. 5026. ISRA/ URV – LNERV/FEVRIER: 2-25.

Kroger, M. (1971**)**, Instrumental Milk Fat Determination. I. Effects of Potassium Dichromate Concentration and Sample Storage Tank on Milko-Tester Results. J. Dairy Sci. *54* (5), 735-737.

Kroger, M. (1985) Milk Sample Preservation. J Dairy Sci.,*p, 68*, 783-787.

Kurzbaum, A., Safori, G., Monir, M., and Simsolo, C. (2008). Anticholinergic syndrome in response to lupine seed toxicity. *Israeli Journal of Emergency Medicine* **8**, 20-22.

Lacroix, C., Verret P. and Paquin, P. (1996). Regional and seasonal variations of nitrogen fractions in commingled milk. Int. Dairy J., 6: 947-961.

Lindmark-Mansson, H. Svensson, U. Paulsson,M. Alden,G. Frank B. and Johnson, G. (2000). Influence of milk components, somatic cells and supplemental zinc on milk process ability. Int. Dairy J., 10: 423- 433.

MAR (2007). Ministry of Animal Resources. Dairy production in Sudan, report.

Malbe, M., Otstavel, T., Kodis, I., Viitak, A. (2010): Content of selected micro and macro elements in dairy cows'. *Agronomy Research* 8 (Special Issue II), 323-26.

MAR (2008). Ministry of Animal Resources. Dept. of statistic information, Khartoum – Sudan.

Martindale, W. (1996). Martindale: The Extra Pharmacopoeia, 31st ed. Royal Pharm. Soc., London, p, 1370.

Martínez-Villaluenga, C., Frías, J., and Vidal-Valverde, C. (2006a). Functional lupin seeds (Lupinus albus L. and Lupinus luteus L.) after extraction of α- galactosides. *Food Chemistry* ***98****, 291-299.*

Martínez-Villaluenga, C., Zieliński, H., Frias, J., Piskuła, M. K., Kozłowska, H., and Vidal- Valverde,C. (2009). Antioxidant capacity and polyphenolic content of high-protein lupine products. *Food Chemistry* **112**, 84-88.

Mather, I. H. and Keenan, T. W. (1998). Origin and secretion of milk lipids. *Journal of Mammary Gland Biology and Neoplasia 3*, 259-273.

McDowell, R. E., (1972). Improvement of livestock production in warm climates. San Francisco: W. H. Freeman and Company.

Minzner, R.A.and Kroger. M. (1974), Physicochemical and bacteriological aspects of preserved milk samples and their effect on fat percentage as determined with the Milko-Tester. J. Milk Food Technol., *37*, 123.

Muir, D.D. (1992). Milk chemistry and nutritive value. In Early, R. (ed) The Technology of Dairy Products.VCH Publishers, Inc., New York, p, 24-33.

Murphy, S. C. and Boor, K. J .(2000).Trouble – shooting sources and causes of high bacteria counts in raw milk , Dairy Food and Environmental Sanitation ,20 (8) : 606 -611.

Ng-Kwai-Hang K. F.and Hayes J. F. (1982). Effects of potassium dichromate and sample storage time on fat and protein by Milko-Scan and on protein and casein by a modified Pro-Milk Mk II method. *J. Dairy Sci.* 65: 1895–1899.

O´connor, C. B. (1995).Rural Dairy Technology. International livesock Research institute, Addis ababa ,Ethiopia.

Olson,J .C. and Mocquat, G.(1980).Milk and milk products , In:Microbial Ecology of Foods , Vol .LL. J.H. Silliker, R. R. Elliott, A.C. Baird parker, F. L. Bryan, J. H. Christion , D.S. Clark J C .Olson ,and T.A. Roberts (ed). Academic press, N.Y.P.470 – 520.

Ollivier-Bousquet, M. (2002). Milk lipid and protein traffic in mammary epithelial cells: joint and independent pathways. *Reproduction Nutrition Development 42*, 149-162.

Ozer, B.; Grandison, A.; Robinson, R. and Atamer, M. (2003). Effects of lactoperoxidase hydrogen peroxide on rheological properties of yoghurt. J. Dairy Res., 70: 227- 232.

Padhye, N. V. and Doyle, M. P. (1992).*Escherichia coli* epidemiology, pathogenesis and methods for detection in food . J. Food Prot., 55(7):555 - 556.

Pastor-Cavada, E., Juan, R., Pastor, J. E., Alaiz, M., and Vioque, J. (2009). Analytical nutritional characteristics of seed proteins in six wild Lupinus species from Southern Spain. *Food Chemistry* 11**7**, 466-469.

Payne, W. J. A. and Hodges, J. (1997). Tropical cattle. Cambridge: The University Press.

Rea, M. and Fleming, M. (1994).Escherichia coli.In:The significance of pathogenic microorganism in raw milk.Interational Dairy Federation (IDF),Docoment No.292.Belgium.

Roux Y, Guinot-Thomas P, Colin-Schoelleno and Laurent F, (1995). Protéolyse et qualité du lait. Colloque National (filière lait, système qualité et certification) INPL- université henry Poincaré: pp. 28 – 29.

Rochfort, S., and Panozzo, J. (2007). Phytochemicals for Health, the Role of Pulses. *Journal of Agricultural and Food Chemistry* **55**, 7981-7994.

Ruiz, M.A. and Sotelo, A. (2001). Chemical composition, Food nutritive value and toxicology evaluation of Mexican wild lupines. J. Agric. Food Chem., 49: 5336.

Saha B. K, Ali M. Y, Chakra borty M, Islam Z and Hira A. K. (2003). Study on the preservation of raw milk with hydrogen peroxide (H2O2) for rural dairy farmers. Pakistan Journal of Nutrition, 2: 36–42.

Sandhu, J.S.; Nusrath, N.; Narayanaswamy, M, and Kanpur, O.P. *(***1984)**, Study on the effect of formalin as preservative on different constituents of raw milk samples during storage. J. Food Sci. Technol. *21* (6), (424-425.24).

Schalm, D.W. Carroll E J, Jain C: Bovine Mastitis. Lea and Febiger: Philadel phia; 1971.

Schrodes, M.J.A. (1982). Effect of oxygen on the keeping quality of milk, I. Oxidized flavor development and oxygen uptake in relation to oxygen availability, J. Dairy Res. (49): 407– 424.

Seskena, R.; Janevica, L. (**2007)** Influence of chemical preservatives on the quality and composition indices of raw milk samples. *723*, 171-180.

Smart, W.L., Raplh, M.M. Lidale, J.L. Ramma, R.D. Robinson, C.J. and Armstrong.E.W. (1988). Looking at Lupines Published at Department of Agriculture Lake Grace.

SPSS v.16.0 Inc (2007). Brief Guide (http:/ www.spss.com).

St-Gelais DS, Haché Gros- and Lois (1992). Combined defects of temperature, acidification, and diafiltration on composition of skim milk Retentate and permeate. J. Dairy. Sci. 75(5):1167–1172.

Sudanimals, (2006). Sudanese cattle. http://www.Sudanimals.com/

Tolle, A .(1981).The bacteriological quality of raw milk. J.Dairy Sci Abstract. 44 No 8593.

Uzun, B., Arslan, C., Karhan, M., and Toker, C. (2007). Fat and fatty acids of white lupin (Lupinus albus L.) in comparison to sesame (Sesamum indicum L.). *Food Chemistry* 102,45-49.

Walstra, P. (1974). High-melting triglycerides in fat globule membrane - Artifact. *Netherlands Milk and Dairy Journal 28*, 3-9.

Walstra, P. (1985). Some comments on the isolation of fat globule membrane material. *Journal of Dairy Research 52*, 309-312.

Walstra, P., Geurts, T. J., Noomen, A., Jellema, A., van Boekel, M. A. J. S. (1999). *Dairy technology: principles of milk properties and processes.* Marcel Dekker. New York, USA. 107-147.

Wolko, B., Clements,J.C. Naganowska,B. Nelson, M.N. and Yang,H. (2011). *Lupines*. In: Kole, C. (Ed.), Wild Crop Relatives: Genomic and Breeding Resources. Legume Crops and Forages. Springer, Berlin, pp. 153-206.

Yanez, E., Ivanoviæ, D. Owen, D.F. and Ballester D. (1983). Chemical and nutritional evaluation of sweet lupines. Ann. Nutr. Metab. 27: 513-520.

Yuan, J. (2001). MAP for shelf – life extension and its synergy with Ozone. Extended shelf life of foods: Quality and Safety Symposium, Seven. Oak Brook, IL.

Zdunczyk, Z., Juskiewicz,J. Frejnaged,S. Flies, M. and Godycka,I. (1994). Chemical composition of the cotyledons and seed coat and nutritional value of whole and hulled seeds of yellow lupine. J. Anim. Feed Sci., 3: 141-148.