Sudan University of Science and Technology
College of Animal production Science and Technology
Animal Production Department (General and Dairy)

Evaluation the quality of Raw Cow's Milk, in the Dairy Farm of College of Animal production Science and Technology

تقييم جوده اللبن الخام لأبقار مزرعة كليه علوم وتكنولوجيا الإنتاج الحيواني

A dissertation Submitted in partial Fulfillment for the Requirements of the (B.sc)

By:

Eman Ahamed Mohammed Mohammed

Hamoda Alazirig Mohammed Adam

Mugtaba Seif Eldein Adam Mohmmed

Supervisor:

Prof. Omer Ibrahim Ahmed Hamid

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الاستهلال

بِسۡمِ ٱللَّهِ ٱلرَّحۡمَٰنِ ٱلرَّحِيمِ

قال تعالي:

﴿ وَإِنَّ لَكُمۡ فِي ٱلۡأَنۡعَٰمِ لَعِبۡرَةٗۖ نُّسۡقِيكُم مِّمَّا فِي بُطُونِهِۦ مِنۢ بَيۡنِ فَرۡثٖ وَدَمٖ لَّبَنًا خَالِصٗا سَآئِغٗا لِّلشَّٰرِبِينَ ﴾

صدق الله العظيم

سورة النحل ،الآية (66) .

Dedication

We dedicate this humble effort, which is the salvation of years of diligence, patience and perseverance in the paths of science and knowledge

To My Mother:

The source of tenderness and the shadow of the sword

To My father:

Who taught me and is my best teacher

To My Dear brothers:

Who are the most precious of hat has given me life.

To My Friends:

Who lived with them beautiful moment and miserable

To:

Everyone who has contributed to my education career

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Abstract

The current research was conducted to study the quality of cow's raw milk produced in the dairy farm of the College of Animal Production Science and Technology. A total of 30 samples of raw milk were collected from bulk milk of dairy farm from morning and evening milking and were then subjected to laboratory tests, including physicochemical (specific density, fat, lactose sugar,**p**roteins, solids nonfat ash) and microbiological (Total bacterial count, total count of, total count of *E.coli*, total count of coliform, total count of *Staphylococcus aureus)* analyses. The results showed that no significant variations in the physicochemical and microbiological quality of the milk. The chemical composition of the raw milk samples was within the normal range. The microbiological contents showed presence of harmful bacteria mainly *E.coli*, coliforms, and *Staphylococcus aureus* therefore no significant differences were observed in the bacterial counts between the morning and evening milk. Its concluded that the raw cow's milk of the College dairy farm microbiologically contaminated with many pathogenic bacteria which indicated bad hygienic conditions during milking.

Keywords: Quality, total bacterial count, pathogenic bacteria, total solid .

ملخص الدراسة

أجري هذا البحث لدراسة جوده لبن الأبقار الخام المنتج بمزرعة ألبان كليه علوم وتكنولوجيا الإنتاج الحيواني . تم جمع 30 عينه لبن خام من أبقار مزرعة الكلية من حلبة الصباح والمساء ومن ثم أخضعت لاختبارات معمليه شملت التحليل الفزيوكيميائي (الكثافة النوعية، الدهن، سكر اللاكتوز، البروتين، الجوامد الحلبة اللادهنيه، والأملاح) والاختبارات الميكروبيولوجيه (العد البكتيري الكلي، عد، الكوليفورم، عد الاشريشيه القولونية، عد العنقودية الذهبية). أوضحت النتائج انه لاتوجد فروق معنوية في جوده اللبن الفيزيوكيميائيه والميكروبيولوجيه . ووجد أن التركيب الكيميائي ضمن ألمدي الطبيعي. والمحتوي البكتيري ضار خاصة الاشريشيه القولونية والعنقودية الذهبية والكوليفورم. كما وجد انه لاتوجد فروق معنوية في العد البكتيري بين حلبتي الصباح والمساء. خلاصه الدراسة إن اللبن المنتج بمزرعة ألبان كليه علوم وتكنولوجيا الإنتاج الحيواني ملوث ببعض البكتريا الممرضة مما يشير إلي الظروف الصحية الغير جيده أثناء عمليه الحلب.

CHAPTER ONE

INTRODUCTION

Milk is legally defined as the normal secretion of the mammary gland of mammals, and cannot be colostrum's or colostrum's milk like (Clarence.et.al.2004).

Cattle Milk can also be defined as the original milk of one or more cows, which has not been heated to more than 40^{o}C, and has not been submitted to any kinds of treatment (Edgar and Axel, 1995).

Milk is a white liquid but it can be slightly yellowish, especially during the summer when the cows are out in the meadow (Eddgar and Axel, 1995).

Milk is an important source of nutrients to human and animals. It is meant to be the first and the only food for the offspring of mammals as is almost complete food (Pandey and Voskuil, 2011). Almost 87% of milk is composed of water and the remaining part comprises total solids (carbohydrates, fat, proteins and minerals) contained in a balanced form and digestible elements for building and maintaining the human and animal body. Other milk ingredients include immuno-globulins which protect the newly born against a number of diseases (Pandey and Voskuil, 2011). Milk has a complex biochemical composition and its high water activity and nutritional value serves as an excellent medium for growth and multiplication of many kinds of microorganisms when suitable conditions exists (Parekh and Subhash 2008).Raw milk is an important vehicle for the transmission of milk-borne pathogens to humans, as can be easily contaminated during milking and handling (Addo*et al.*, 2011). Being highly perishable commodity and highly nutritious food, milk serves as an ideal medium for the growth and multiplication of various microorganisms (ParekhandSubhash,2008). Microbial contamination in milk may cause milk-

borne diseases to humans while others are known to cause milk spoilage. Many milk-borne epidemics of human diseases are spread through milk contamination. Sources of microbial contamination in milk include primary microbial contamination from the infected or sick lactating animal. The secondary causes of microbial contamination occurs along the milk value chain which may include contamination during milking by milkers, milk handlers, unsanitary utensils and/or milking equipment and water supplies used in sanitary activities. Other secondary sources of microbial contamination occur during milk handling, transportation and storage. There is tertiary microbial contamination which occurs mainly due to re-contamination of milk after being processed due to unhygienic conditions and/or poor or improper handling and storage of milk during consumption (Parekh and Subhash 2008).

1.1.Objective of the study:

The main objective of this study is to evaluate the quality of raw cow's milk, in the dairy farm of College of Animal production Science and Technology, Sudan University of Science and Technology.

CHAPTER TWO

LITERATURE REVIEW

2.1.Definition and Composition of Milk

Milk is a yellowish-white non-transparent liquid secreted by the mammary glands of all mammals. It is the primary source of nutrition and sole food for offspring of mammals before they are able to eat and digest other types of food. It contains in a balanced form of all the necessary and digestible elements for building and maintaining the human and animal body (Pandey and Voskuil, 2011). The main composition of milk is water (87 – 88%); the remaining part is total milk solids which include carbohydrates, fat, proteins and ash or minerals. This composition is not constant, the average percentages of milk components vary with species and breeds of animal, season, feeds, stage of lactation and health and physiological status of a particular animal (Pandey and Voskuil, 2011). Sometimes the composition might even change from day to day, depending on feeding and climate, but also during milking the first milk differs from the last milk drops (Pandey and Voskuil, 2011).

2.1.1.Water:

Water is considered to be the largest component of raw milk and in water other milk components are released where the fat is emulsified with most protein and calcium and phosphorus are found to be liquefied or stuck in water. lactose, mineral salts and some vitamins are in the case of the solution with milk water(Board 2007).Water plays an important role in maintaining the place of milk components and is also a medium for many microbiological and auxiliary activities in chemical reactions. According to Spreer(2007) and (Enemir200water is divided to:

2.6.1.1.Bound water:

is defined as the water of the licorice and it has no known water properties This water is attached to the fat grains and is estimated to account for about 4% of the total water content of the milk.

Milk contains 79-89% water (Adam, 2002) while Zalzla (2000) indicated that the percentage of water in human milk and cow ranges between 87-87.5%;

2.1.1.2.Free water:

is known as water can be disposed of by heating the milk at a temperature of 105^{o}C for 2-3 hours under normal atmospheric pressure. The percentage of the free water is about 96% of the total water of the milk. The difference in the percentage of water in the milk a caused by different factors including the preed, dynastic type age animal variation seasonal difference as mentioned by Berbary (2000).

2.1.2.Milk fat:

Milk fat is easy to digest because it is in the form of small granules with a large surface which presents them to rapid enzymatic degradation it contains a high percentage of about 10% of the total fatty acid series (4-10 carbon atom) compared to other animal fat (Enemir2007). The milk fat is made up of three triglycerides and forms about 85% of its composition and the rest is expressed as monoclonal and bivalent glycerides – fatty acid and lipid substances. in addition the milk medium contains the vitamins(A-D-E-K) and contains a high percentage of about 30% of the total fatty acid of of unsaturated fatty acid. as well as a good proportion of 4% of total fatty acid of essential fatty acid (linolenic and linolenic) which cannot be synthesized in the body, this is true for other units such as phospholipids as well as free fatty acid monoclonal and

bivalent glycerides and some waxes as mentioned by Smit (2005),Abudaoud*et.*al.(2003) and Jones(2002). The average fat content in milk is 3.9% (Uguelph2007)between 3.5-6% (Spreer,1995) and3.5% (Bakiri1994).(3.30%)According to Sudanese Standards and Metrology(2007). There are many factors that affect the proportion of fat in raw milk including the type of animal, productive life, milking season, season of the food, period between the milking and the state of the animal at birth and its role According to Al- Hajrawi(1987).

2.1.3. Milk Protein:

The protein is a result of the amino acid union through the bibtide links milk contains on to basic types of proteins with small amounts of other types of multiple as indicated by Suliman (2008) and Goff(1995) and Smit(2005) and the two main types are:

2.1.3.1.Casein:

It is known as the main protein in milk which accounts for 80% of all milk protein and is composed of serval cracks the most important alpha-casein- beta-casein and capa-casein.

2.1.3.2.Whey protein:

Form about 18% of milk protein are the protein left after the deposition of casein and contains several protein the most important are:

β-lactoglobulin.

α-lactoalbumin.

According to Smit (2005) and Abudaoud et.al(2003) milk contains other proteins such as proizepeptone, lactoferrin, lactin and urea, the concentration of proteins in milk is about 2.5-3% according to the Enemir (2007).The Dubai

International Food Safety Conference(2007). gives a percentage of 2.5-4.2%**2.1.4. Milk sugar (lactose)**:

Lactose is the main and only carbohydrate representative in milk .it is called milk sugar and found in all types of milk. unlike lactose there are traces of glucose, galctose and some other sugar Abdullatif(2005).the lactose is a diabetic reductase consisting of glucose and galactose as indicated by both Uguelph (2007) and Aweda(2004) and there real solution on whey which is two types:

β-Lacto globulin

α-Lacto albumin

The percentage of lactose in cow milk is 4.8-5.2% (smit 2005) 4.91% (Abudaoudet al.2003) (4-5% Zalzala2000).

2.1.5. Milk minerals:

There are about 22 types of salts in milk and include three groups according to Sulaiman(2008) and Goff (1995) which are:

Sodium, potassium and chloride which are free ions have a relationship with lactose in order to maintain the equilibrium of the osmotic pressure with blood

Calcium – magnesium –inorganic phosphorus and striate this a group is aphyto form in the cells of casein- diffuse salt such as calcium magnesium citrate and phosphate, The concentration of Ash which equal 0.70-0.80% 0f the weight of milk(Srivastava2010).

2.1.6. Enzymes of milk:

Are proteins compound have an auxiliary role in the biological reaction and are characterized as specialized in their work and that each enzyme of a certain temperature and temperature of the same as the enzyme at the maximum

activity. Milk enzymes have an important effect on the characteristics of milk and milk products and the most important according to Sulaiman(2008).

2.1.6.1. Lipases:

Lipase enzyme splits fat into glycerol and free fatty acids this enzyme is found mainly in the plasma in association with casein micelles(Srivastava2010).

2.1.6.2.Catalase:

This splits the hydrogen peroxide into water and molecular oxygen. This slowly reduces the milk protein and helps in ripening of cheese(Srivastava2010).

2.1.6.3.Phosphatase:lineMilk contain both alkaline(pH 9.6) and acidic (pH 4.0) phosphatase enzymes. Alkaline phosphatase enzymes are able to split specific phosphoric acid esters in to phosphoric acid and the related alcohols(Srivastava2010).

2.1.6.4.Lactase:

Analyzes lactose sugar(Sulaiman 2008).

2.1.6.5.Protease: Analyzes milk protein(Sulaiman 2008).

2.1.6.6.Peroxidase: It is the most abundant enzyme present in milk (more in the buffalo milk than cow milk). Heat treatments of milk such as 80^{o}C for three and one half min., 73.5^{o}C for 28 min. or 70^{o}C for 150 min. destroy this enzyme(Srivastava2010).

2.1.7.Milk vitamins:

Vitamins are essential substances that the body need and contains milk for all the vitamins needed enrich the human, Milk is considered one of the best as source of vitamin A-B2-pantothenic it contains small quantity of vitamin E, B1.The vitamins found in milk are soluble in fat which are vitamin A,D, E, K

and some are soluble in water which is a combination of vitamin B- complex and vitamin C according to Enemir(2007) (Adam2002).Fox and MC Sweeny(1998).

2.2. Nutritional value of milk:

Milk contains fat, protein, carbohydrates, salt, and vitamins in proportion to the needs of the body, making milk the perfect food and the closest to perfection, it calls it full food (Elkholi, 1999).

2.2.1. Milk protein:

Milk proteins contain essential amino acids, which are of high are of a high bio-value and are enriched by the flavorful, complementary and puree and characterized by their cheaper price compared to other animal proteins, proteins is essential for destruction and construction and is easy to digest(Campbell and Marshall,1975).

2.2.2 Milk fat:

Milk contains essential fatty acids necessary for the human body like (linoleic) and (Arachidonic) which help in the absorption of vitamins dissolved in fat. It is easy to digest 98% of which is digested. This is due the fact that milk fat is characterized by high melting point, according to (Hajrawi,1987) milk fat contains some compounds such as phospholipids, carotene and all of these substances generates them some vitamins such as choline and vitamin D, A, the fat milk is different from other animal fats by containing a higher proportion of unsaturated fatty acids(Elkholi, 1995).

2.2.3. Lactose:

milk is only source of lactose sugar in nature, which is less sweet than sucrose sugar. Lactose is especially important in the field of biologic, which is

necessary for the formation of galactose of glucose, which is necessary for the installation of glands and nerve tissue in the first weeks of life .it has the advantage of softening the digestive tract and contributes 30% of the energy produced by the milk. (Murshidy,1995).

2.2.4. Mineral and milk salt:

The minerals that are readily available in milk are calcium and phosphorus, milk is an important dietary source of calcium, it was found that one liter of milk equivalent in its content of calcium 21 eggs or12 kg of meat calves or 20 kg of whole wheat bread according Roberts(2007).

2.2.5 milk vitamin:

Vitamins are substances that the body needs in very small quantities and is essential for metabolism and is important in nutrition because it protects the body from many malnutrition diseases and thus helps to grow healthy. It has been found that the human body does not have the ability to from the vitamin, but it is taken from different foods and it is very rare to meet all the essential vitamins in one diet, but milk is characterized by containing most vitamins known as vitamins B2, B1, A. milk is also a major source of vitamin E and a poor vitamin D milk is not an Important source of vitamin(Khuli1995).

2.3. Milk properties:

The properties of the chemical milk play an important role in estimating its quality and milk quantity addition to preservation and processing.

2.3.1.Specific density:

The density of the water is equal to one at temperature 4^{o}cwhile the density of the milk more than one to contain solid materials. The quantity varies according to quantity of solid materials and the temperature of the milk(Murshidy1995).

As well as the Enemir (2007) that the density of the milk ranged between 1.030-1.036 at a temperature 15.5^{o} C and increase the content of the cream in the milk less weight specific (Hurly2009) and (Mohammad *and et al*. (2000) and (Elkhuli1995) indicated that the density of milk was averaged as follows (1.027-1.033) and (1.028-1.034) and (1.032) at 15 respectively. Milk density increases with the addition of water and increases when removing fat or adding solid material such as flour (board 2007).and 1.029 According to(SSMO) Sudanese Standards and Metrology Organization (2007).

2.3.2.Acidity:

The percentage of acid in fresh milk ranges from 0.12-0.16% with an average of 0.14% on the basis of lactic acid and called natural acids and is caused by protein, phosphates, jackets and carbon dioxide (murshidy 1995). (Enimir (2007) said the acidity rises a result of lactic acid bacteria that that consume lactose sugar and if the degree of acidity is higher than 0.20% known as advanced acidity.

2.3.3. pH: The hydrogen number of raw milk range with a mean of 6.65-6.66 according to Murshidy(1995) and 6.4-6.8 guides according to Al-hajrawi (1987).**2.3.4.Color:**

According to Murshidy(1995) the color of milk varies between white glaucoma and white which is yellowish depending on the animal variety type of cut quantity of fat and solid matter in milk. the white color of the milk is due to the reflection of the light on the molecule of casein and calcium phosphate and the grain of fat spread and the yellow color in milk to the presence of dye carotin lupus in fat .

In the event of any change in the milk from the above color this refers to the non-color nature due to different factor(Enemir2007).

2.3.5.The taste:

It is not possible to determine the taste of raw milk as it has a light or salty flavor is hardly appear with the not that the sugar lactose and chloride salts are the main responsibility for the presence of that test(Asia2017).

2.3.6. The smell:

For raw milk smell disappear after hours or cooling but it is highly efficient to absorb smells of barns or some smells that may reach the milk through some of the delicious which is fed the animal (Murshidy1995) and (Zedan2004).

2.4.Qualityand quality control:

Overall quality is to make the product more capable of outstanding performance in the market or meet market requirements in terms of design good performance and after sales service. The American quality standards institute defines the characteristics and features of a product or service that make it capable of meeting certain requirements(Mustafa(2008).

2.4.1. The method used in determining quality:

The methods used to determine quality can be divided according to El-Sayid(1998)into:

2.4.1.1. Sensory methods:

Sensory method of quality assessment are accredited researchers and are created as a result of previous practice individual experience and strong recognition.

2.4.1.2. Mechanical methods :

It is based on influenced by researcher’s position and divided into three section:

2.4.1.2.1. Physical methods of measurement

This method is one of the best method to do it. It does not need very special people.

2.4.1.2.2. Chemical methods of measurement:

In general, chemical methods for dairy analysis are used to estimate food values properties and quality levels.

2.4.1.2.3. Microbial methods:

The microscopic methods are very important in terms of their application in quality control programs but require experienced people to interpret the results.

2.5. Hygiene, Handling and Microbial Quality of Raw Milk

Milk is a perishable product and an ideal medium for the growth of a wide variety of bacteria (Parekh and Subhash, 2008). When it is secreted from a healthy udder, raw milk contains only a very few bacteria of about 500 to 1,000 bacteria per milliliter (Omoreetal. 2005; Pandey and Voskuil, 2011). After milking environmental contamination occurs, which in turns increases the total bacteria count up to 50,000 per ml or may even reach several millions bacteria per milliliter (Pandey and Voskuil, 2011). That count level indicates a very poor hygienic standard of milk during milking and handling or milk of a diseased animal. The presence of coliform bacteria particularly *E.coli* in raw milk is an indicator of feacal contamination which implies poor hygienic conditions and unsanitized environment since these bacteria are of faecal origin. (Pandey and Voskuil, 2011).

2.6.Pathogenic bacteria in raw milk:

2.6.1.*Staphylococcus aureus*:

Staphylococcus aureus is an important food bornepathogen and causes a mild skin infection to more severediseases, such as pneumonia and septicemia (Lowy1998). *Staphylococcusaureus* is a major causative pathogen of clinical and subclinical mastitis (Adwan2005)Milk has been reported as a common food that may cause staphylococcal poisoning (Le loir,2003)*Staph. Aureus in* milk should be regarded as a part of the risk analysis of milk(Zouharova2005).

2.6.2.Coliform:

Coliform are almost always found in raw milk but with good methods of production number of Coliform can be kept very low (Boor et al., 1998). The presence of these organisms in milk and milk products is an indication of unsanitary production and or improper handling of either milk or milk utensils (El-zubeir and Ahmed, 2007). Milking udder with sub-clinical mastitis and wet environment lead to contamination of bulk tank milk and hence raw milk reaches the consumers with elevated Coliform count (FAO, 2008; Zadoks et al., 2007). Kagki *et al.* (2007) showed that in addition to faecal contamination, other factors such as milking wet udders, inadequate cooling of milk and udder infection are the main sources of Coliform in bulk milk. College of Agriculture and Life Science (2001) asserted that Coliform are associated with fecal and environmental contamination. Coliform count of less than 100 cell/ml is considered acceptable, but count of less than 10 cell/ml is achievable and desirable (Boor et al., 1998). Coliform count above 500 cell/ml indicates poor hygiene either during equipment cleaning or between milking with common contaminants such as bedding, manure, soil or water (Murphy and Boor, 2003)Bulk milk Coliform bacteria are used as indicator of hygienic condition during handling and processing of milk and milk products (College of Agric and

life Sciences, 2001). In addition to the use of solid media from which the Coliform density can be counted the use of liquid media which can be worked out by the most probable number (MPN) can be used (Messer and Dufour, 1998).

2.6.3. *E. coli*:

E.coli is oftenused as marker organisms. Recovery and counting of *E.coli* is used as reliable indicator of fecal contamination and indicates a possible presence of enteropathogenic and/or toxigenic microorganisms which constitute a public health hazard. *E. coli* is one of the main inhabitants of the intestinal tract of most mammalian species, including humans and birds. Most *E. coli* are harmless, but some are known to be pathogenic bacteria, causing severe intestinal and extra intestinal diseases in man (Kaper*etal*., 2004). *E.coli* is frequently a contaminating.

2.6.4.Total bacterial count:

The total initial number is less than 1000 cuf/ml where contamination during production is as low as any more than 100000 cuf/ ml milk a record number of more than 100000 cuf\ml indicates a plan to apply health requirements while milk production in less than 10000 number reflects good health practices. Al-muhaiza *et. al* (1997)$5x10^5$cuf\ml According to Sudanese Standards and Metrology Organization(2007).

2.7. Sources of contamination in milk:

Milk is sterile when it is in the udder of a health animal but becomes contaminated with bacteria mainly during and/or after milking (Karimuribo *et al*., 2005; Makerere University, 2011). Milk from subclinical mastitic cows usually contains bacteriological agents but milk from non-mastitic cows is often contaminated from extraneous dirt or poor quality water (Kivaria*et.al*.2006).

Microbial contamination in milk comes from milk itself as it can be naturally contaminated or comes from infected or sick animal, human, environment, water and equipment used for milking and storage of milk. These sources of contamination include disease-causing organisms (pathogens) shedding in milk, infected udder and/or teats, animal skin, faecal soiling of the udder, contaminated milking and storage equipment and water used for cleanliness. Other bacterial sources are from air, 9 milkers, handlers, drugs or chemicals used during treatment of animal and from water used for adulteration by unscrupulous and unfaithful workers/sellers which may be contaminated and may cause additional health problems (Karimuribo *et al.*, 2005; Swai and Schoonman, 2011). Exposure of milk to these sources or conditions may lead to increased microbial contamination and affect its quality. Although, sometimes re-contamination may occur after processing and is mainly due to unhygienic conditions, poor or improper handling of milk during consumption (Parekh and Subhash, 2008). In general quality of milk may be lowered when it is contaminated by a number of factors such as adulteration, contamination during and after milking, presence of udder infections, mastitis (inflammation of mammary gland) disease and drugs residues used for treatment of disease which is considered to be public health concern and one of the most important causes of economic losses in the dairy industry worldwide (Karimuribo *et al.*, 2005; Syit, 2008; Mdegela *et al* 2009)

.2.8. Grading of raw milk:

Raw milk under tropical condition was graded according to many factor which include the number of microorganisms present in it, odor or flavor, amount of sediment appearance and temperature (Chamdan and Hedrick, 1979). (John and Robert1975), (William and pual1980) , and Sudanese Standards and Metrology Organization (2007), reported that milk was graded as a good when it had total bacterial count (TBC) of 5.0 $x10^5$ cuf\ml or less , satisfactory when the (TBC)

ranged between 5 $x10^5$ to $5x10^6$ cuf\ml and bad when the (TBC) was more than $5x10^6$cuf\ml. and the U.S standard classified or grading the quality of milk in to graded:

Grade (A) when it had TBC less than $2.0x10^4$cuf\ml

Grade (B) when had TBC ranged between $2.0x10^4$ to$1.0x10^6$cuf\ml

Grade (C) when it had TBC less than to $1.0x10^6$cuf\ml (U.S department of Health Education and Welfare1953).

CHAPTER THREE

MATERIALS AND METHODS

3.1.Study area:

The study was conducted at the College of Animal Production Science and Technology - Sudan University of Science and Technology - Kuku during the period 4 March to 19 March 2018.

3.2. Sampling of milk:

A total of 30 samples of raw cow milk were collected from the cow's milk from the farm of the College of Science and Technology of Animal Production (Morning and Evening Milk).Fifty ml milk samples were collected directly from the milking containers into a sterile screw tubes.

3.3. Milk Sample handling

All samples were coded with numbers for identification and stored in a icebox.

3.4. Laboratory Analysis of Milk Samples:

Samples were analyzed in the microbiological laboratory of the College of Veterinary Medicine and Animal Production. Two kinds of laboratory analyses of milk samples were performed. First was analysis for microbiological of raw milk included (Total bacterial counts (TBC),Coliform count, *E.coli* count, *Staphylococcus aureus* count). The second analysis was chemical composition of raw milk.

3.5. Determination of microbial quality of raw cow milk

3.5.1. Bacterial count:

Common bacteria reported to be isolated from milk include total bacterial count, Coliform count, Salmonella count, *E. coli* count, *St. aureus* count.

3.6. Materials:

3.6.1.Equipment:

penzin burner, Sensitive balance, Incubator, Lope, Petri dishes, Sterile oven, Conical Flask, Sterile Pipette, Tips, Sterile Test tubes**,** Autoclave.

3.6.2.Chemicals:

Normal saline, Alcohol, Distill water.

3. 7. Media preparation and storage:

All the media used in this study were prepared according to manufacturer's instructions. of the preparations and handling of different types of media used is hereby shown:

3.7.1. Nutrient Agar (NA)

Meat extract 3.0 g
Peptone 5.0 g
Agar 12 g to 18
Water 1000 ml

Preparation:

Dissolve the dehydrated medium in the water by heating if necessary. Adjut pH to ~7.0 after sterilization, transfer into bottles and autoclave at 121 ^{o}C for 20 min. Pour 15 ml of melted medium in each plate.

3.7.2. Violet Red Bile Glucose (VRBG)

Yeast extract, 3.5g\l

Peptone 7g\l

Sodium 5g\l

Chloride 1.5g\l

Bile salts No. 3.10g\l

Glucose, 0.03 g/l

Neutral red, 0.002 g/l

Crystal violet, 12 g/l

Agar 23

pH 7.4 ± 0.2 at25°C.

preparation:

It was prepared according to the manufacturer's instructions whereby 38.5 g of the powdered medium was suspended into 1 liter of distilled water. The medium was boiled for 1 minute with frequent agitation to dissolve completely. No further sterilization is necessary. Then, was mixed well and placed into water bath set at 48ºC for use within 3 hours from preparation time.

3.7.3.Eosin Methylene Blue Agar:(E.M. B):

Composition:

Ingredients Gms/ Litre

Peptic digest of animal tissue	10.000
Dipotassium phosphate	2.000
Lactose	5.000
Sucrose	5.000
Eosin – Y	0.400
Methylene blue	0.065
Agar	13.500
Final pH (at 25°C)	

Preparation:

Suspend 111.02 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs. pressure (121°C) for 15 minutes. Cool to 45-50°C. If desired, add 5% v/v Egg Yolk Emulsion (FD045). Mix well and pour into sterile Petri plates.

3.8. Normal saline solution:

The solution was prepared by dissolving 0.85 g of Sodium chloride (Sigma-Aldrich, Co., USA, Cat. S5886, Lot SLBC3215V) into 100 ml of sterile distilled water, mixed well and sterilized by autoclaving at 121°Cfor 15 minutes and cooled to below 45°C, the solution was ready for use.

3.9.Standard plate count (SPC):

The total bacterial count was made by adding 1 ml of milk sample into sterile test tube having 9 ml peptone water. After thoroughly mixing, the sample was serially diluted up to $1:10^{-7}$ and duplicate samples (1 ml) were pour plated using 15-20 ml standard plate count agar solution and mixed thoroughly. The plated

sample was allowed to solidify and then incubated at 30ºC for 48 hours. Colony counts were made using colony counter (Mirth 1978).

3.10. Coliform count (CC):

One ml of milk sample was added into sterile test tube having 9 ml peptone water. After mixing, the sample was serially diluted up to 1: 10^{-5} and duplicate samples (1 ml) were pour plated using 15-20 ml Violet Red Bile Agar solution (VRBA). After thoroughly mixing, the plated sample was allowed to solidify and then incubated at 30ºC for 24 hours. Finally, colony counts were made using colony counter (Marth 1978).

3.11.*E. coli* counts(EC):

Brilliant green lactose bile (BGB) broth (Merck, 736) and peptone water (Himedia, M028) were used for enumeration of *E. coli* most probable number per ml (MPN/ml). MacConkey broth tubes positive in the total coliform counts were gently agitated and one loopful from each was transferred to a tube of BGB broth and an other loopful to a tube of peptone water (Tryptone water). Both kinds of tubes were incubated in Astell Hearson incubator or Thermoregulation water bathfor 24 – 48 hours at 44.5^{o}C. After 24 hours of incubation 0.2 ml of Kovac's reagent was added to each tube of peptone water, shaken and left to stand for 10 minutes for indole production. Tubes of BGB broth were examined for turbidity and gas formation in Durham tubes. Positive results were used as indication of presence of E. coli. The positive tubes of BGB broth were used for further confirmation of the presence of *E. coli* by streaking a loopful from each tube on Eosin methylene blue agar (EMB) for identification of colonies which show nucleated dark center with or without metallic sheen which are characteristic features of growth of *E. coli* in the medium. The isolates were further characterized by biochemical tests according to Cowan and

Steel (1993). Special attention was paid to the pattern of reactions of the organism in IMVIC tests. *E. coli* most probable numbers per ml of sample were calculated from the number of positive tubes of BGB broth and peptone water (Thatcher and Clark, 1968; Marshall, 1992).

3.12.*Staphylococcusaureus* counts:

Mannitol salt agar (Scharlau,1967) was used for the enumeration of coagulase positive Staphylococci, 0.2 ml quantities of each sample decimal dilutions, 10^{-3} and 10^{-4} were streaked in duplicates in dried plates of Mannitol salt agar. The cultures were incubated at 37^{o}C for 48 hours (Harrigan and McCance, 1976; Raymane*t.al*,1988). Colonies of *Staphylococcus aureus* were recognized by bright yellow zones formation in Mannitol salt agar (Jawez and Adel, 1990). They were counted by colony counter. Confirmation of identified organisms for morphological and staining characteristics were carried out by microscopic examination of Gram's stained smears and coagulase test.

3.1,4. Counting of bacterial colonies:

After the incubation period, bacterial colonies on the culture plates were countered manually. Two critical dilutions per each sample were counted. A plate was divided into quarters using a marker-pen and colony forming units were counted on at least two critical dilution plates by the aid of colony counter. Two consecutive plates with less than 300 colonies were considered for record (ISO 4833-1:2013).

3.15.Determination of chemical quality:

3.15.1. Lacto scan or milk analyzer:

21.625. Egyp.

CHAPTER FOUR

Results and Discussion

4.1.Chemical composition of the cow's milk

4.2.1. Specific density:

The results showed (Table1),that there was no significant difference($P<0.05$) in the Specific density between the morning and evening cow's milk.. The average specific density of milk samples was 1.027±0.00 this result was inagreement with that by Hurly(2009) and Mhmmed*et.al.(*2000) was not in line with Elkoli(1999).

4.2.2.Fat:

The results showed(Table1), that there was no significant difference in the fat contents between the morning and evening cow's milk. The average fat content was 4.20±0.95%this result was in accordance with that by Spreer (1995). And above the fat content provided by Uguelph (2007), Bakiri*et.al* 1994).

4.2.3. Lactose sugar

The results showed(Table1)، that there was no significant difference in the lactose sugar between morning and evening cow's milk. The %of lactose sugar was4.20±0.95 % this result was not in agreement of that provided by Abudoud *et al*(2003) and Smit (2005) and it isin the range provided by Zalzlah (2000).

4.2.4 Proteins:

The result showed(Table1), that there was no significant difference in the ratio of protein in the morning and evening milk, and the average ratio of protein is3.05±0.16% , that is not identify with the percentage attachment by the Enemir

(2007),while in agreement with the value provided by Dubai International Food Safety Conference,(2007).

4.2.5.Solids nonfat:

The result showed(Table1), that were no significant difference between the average fat content in the morning and evening milk, the average of solids nonfat was 8.30±0.25%, that percentage is in line withthe Sudanese Standards and Metrology Organization (2007), which indicated the absence of factors affecting the chemical composition.

4.2.6. Ash:

The results(Table1), showed that there were no significant differences between the average ash contents between the morning and evening milk, the ash content was0.66±0.04%, This value are less than that by Al- muhaiza *et al.*(1997) and Alsayid(2003).Therefore it was in agreement with Srivastava (2010).

Table (1) chemical composition of the morning and evening cow's milk:

Treat.	Fat %	Lactose%	Protein%	SNF%	Ash %	Density
Morning milk	4.20±0.95	4.50±0.12	3.05±0.16	8.30±0.25	0.66±0.04	1.027±0.00
Evening milk	4.10±0.51	4.40±0.27	3.00±0.17	8.40±0.34	0.63±0.01	1.027±0.06
Sig	NS	NS	NS	NS	NS	NS

4.2. Microbiology of the raw cow's milk(Table2)

4.2.1.Total Bacterial Count:

The average total bacterial count of the raw cow's milk of the College dairy farm was4.50±0.33cuf\ml this count was less than that by Alsayid (2003), and Al-muhaiza *et al.* (1997).

4.2.2.*E. coli* count:

The result of statistical analysis showed that there was no significantdifference in the average of *E. coli* count between morning and evening milking, the average of total count of *E.coli*was3.40±0.26 cuf\ml, presence of *E.coli* in raw milk of the College dairy farm probably due to above mentioned factors unhygienic conditions.

4.2.3. Coliform count:

The data revealed high count of Coliform bacteria in the raw cow's milk and no significant differences were found between the morning and evening milk. The average of count of Coliform was3.30±2.60cuf\ml.,

4.2.4. *Staphylococcus aureus* count:

The result of showed that there was no significant difference in the average count of S. aureus count between morning and evening milking, the average of S. aureus count was3.70±3.70cuf\ ml, presence of S. aureus in raw cow's milk of College dairy farm may be due to bad handling and unhygienic milking conditions.

Table (2) Microbial contents of morning and evening milk.

Microbial contents	**Morning milk(cfu/ml)**	**Evening milk(cfu/ml)**
E.coli	3.40±0.26	3.10±0.27
Coliform	3.30±2.60	3.10±2.50
Staphylococcus aureus	4.20±3.40	3.70±3.70
Total count	4.50±0.33	4.40± 0,19
Sig	Ns	Ns

CHAPTER FIVE

Conclusion and Recommendation

5.1. conclusion:

- The study showed that the chemical composition of milk of College dairy farm was in the range of good quality milk no variations were found between morning and evening milk.
- The study showed that pathogenic bacteria was found in the raw cow's milk of the College dairy farm mainly *E.coli* and *Staphylococcus aureus.*

5.2. Recommendation

- ❖ Further studies will be needed to identify yeast and molds in raw milk.
- ❖ Hygienic milking conditions must be followed in milking dairy cows for the safety of the consumers.
- ❖ Wearing protective clothing and clean.
- ❖ Cleaning the Equipment of milking.
- ❖ Cleaning the udder before and after milking.
- ❖ Cleaning milking places.
- ❖ Control to the flies and insects.
- ❖ Cleaning the ground of barns.
- ❖ Cooling the milk immediately after milking.
- ❖ Preventing the consumers and visitor from entering milking place.
- ❖ Activation Machine Milking Technology.

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