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Sudan University of Science and Technology

College of Agricultural Studies



Department of Food Science and Technology

**Microbial Quality Of Cow`s Milk From Different Sources in
Khartoum North Locality**

الجودة الميكروبية للبن الأبقار من مصادر مختلفة في محلية بحري

A dissertation submitted to Sudan University of Science and Technology in partial fulfillment for the requirements of the degree of B.Sc. (Honours) in food science and technology

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الآية

قال تعالى:

(فَتَعَالَى اللَّهُ الْمَلِكُ الْحَقُّ وَلَا تَعْجَلْ بِالْقُرْآنِ مِنْ قَبْلِ أَنْ يُقْضَىٰ إِلَيْكَ
وَحْيُهُ وَقُلْ رَبِّ زِدْنِي عِلْمًا)

صدق الله العظيم

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DEDICATION

*To our families who care about us
to arrive this stage, to our
supervisor, to everyone learns
letter in this life*

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Abstract

This study was carried out to evaluate microbial quality of raw cow's milk taken at different sampling points from farmers, groceries and milk vendors in Khartoum North. The results were as follows frames total viable count of bacteria was 3.65 ± 0.03 cfu/ml, yeasts and moulds 0.897 ± 0.03 cfu/ml, *staphylococcus aureus* 1.18 ± 0.26 cfu/ml, total coliforms 5.11 ± 0.60 MPN/mL, *E. coli* 1.11 ± 0.19 MPN/ml, *Salmonella* was not detected in the modern farm while detected in traditional farm. Groceries total viable count of bacteria was 4.78 ± 0.92 cfu/ml, yeasts and moulds 0.94 ± 0.63 cfu/ml, *staphylococcus aureus* 2.05 ± 0.84 cfu/ml, coliforms 18.5 ± 0.97 cfu/ml, *E. coli* 1.89 ± 0.27 cfu/ml, *Salmonella* was not detected in the supermarkets while was detected in traditional shops and all vendor. Milk vendors total viable count of bacteria was 6.47 ± 0.97 cfu/ml, yeasts and moulds 2.95 ± 0.97 cfu/ml, *staphylococcus aureus* 3.40 ± 0.73 cfu/ml, coliforms 49.1 ± 13.4 MPN/mL, *E. coli* 15.00 ± 3.41 MPN/mL. These result revealed that the microbes less pollution compared groceries this may be due to the milk man or good practices for milking on the farm. The milk vendors are the most contaminated samples this may be due to the milking environment or pots and milk, as well as the movement of vehicle traveling salesman milk it makes it more susceptible to microbes that may comes from dust and frequent conquest of pots and milk and there for entry of microbes.

ملخص الدراسة

هدفت هذه الدراسة لمعرفة الجودة الميكروبية للبن الابقار في محلية بحري حيث تم جمع عينات من لبن الابقار من ثلاثة مصادر مختلفة وهي الباعة المتجولين والمزارع والبقالات الحديثة. و التحاليل الميكروبية للعينات وجدت كالآتي : المزارع العدد الكلي للميكروبات 3.65 ± 0.03 cfu/ml والخمائر والاعفان 0.897 ± 0.03 cfu/ml والاستافيلوكوكس اورييس 1.18 ± 0.26 cfu/ml والكلوروفورم 5.11 ± 0.60 MPN/mL وال *E.coli* 1.11 ± 0.19 MPN/mL والسالمونيلا توجد بنسبة بسيطة . اما البقالات العدد الكلي للميكروبات 4.787 cfu/l, والخمائر والاعفان 0.94 ± 0.92 cfu/ml والاستافيلوكوكس اورييس 2.05 ± 0.84 cfu/ml والكلوروفورم 18.57 ± 0.97 MPN/ml وال *E.coli* 1.89 ± 0.27 MPN/ml والسالمونيلا توجد بنسبة ضئيلة. والباعة المتجولين العدد الكلي للميكروبات 6.47 ± 0.97 cfu/ml والخمائر والاعفان 2.95 ± 0.97 cfu/ml والاستافيلوكوكس اورييس 3.40 ± 0.73 cfu/ml والكلوروفورم 49.1 ± 13.4 MPN/ml وال *E.coli* 15.0 ± 3.41 MPN/ml والسالمونيلا توجد بنسبة كثيرة. حيث كشف التحليل أن الميكروبات في عينات المزارع اقل تلوثا مقارنة بالبقالات قد يعزى ذلك للحلاب او الممارسات الجيدة للحلب في المزرعة أما عينات الباعة المتجولين هي الاكثر تلوثا قد يعزى ذلك للحلاب او بيئة الحلب او اواني الحليب وايضا تنقل البائع المتجول بعربة الحليب يجعله اكثر عرضة للميكروبات التي قد تأتي من الغبار والفتح المتكرر لاوني الحليب وبالتالي دخول الكثير منها للحليب.

CHAPTER ONE

INTRODUCTION

Milk is a nutrient fluid produced by the gland of many mammals for the nourishment of their young. This liquid contains proteins, fat, lactose, various vitamins and minerals. Milk and dairy products are particular good source of many nutrients like calcium which is essential for bone growth; a source of conjugate linoleic acids, fatty acids inhibits skin cancer, colon cancer and breast cancer. One glass of milk alone can make significant contribution to daily recommended intake of many important nutrients for all age groups (FAO, 2002). People in Africa use milk from cows, sheep, goats and camels, and of these sources cow's milk is the most widely produced and processed (FAO, 1990). It is a complex fluid containing a mixture of carbohydrates, protein, fat and minerals in different physio-chemical status and forms (FAO, 1997). Its comprehensive nutritional properties and high moisture content make it an excellent medium and favorable environment for supporting microbial growth resultant spoilage of the product or infections/ intoxications in consumers (O Connor, 1995; Murinda *et al.*, 2004; Oliver *et al.*, 2005). Microbes can enter milk via the air, water and feed (Eberhart, 1977), milk handling, equipments, milkers or during transportation (Freedman, 1977) or directly from dairy cows experiencing sub clinical or clinical mastitis (Rodojcic-Prodaova and Necev, 1991).

Markets and consumers for raw milk and their products have existed in many parts of the world. Raw unpasteurized milk is consumed directly by a large number of people in rural areas and indirectly by a much larger segment of the population via consumption of several types of cheeses. Among the main reasons that people may believe that the raw milk and their products have advantages or value over the pasteurized one (Banwart, 1989; Gruetzmacher

and Bradley, 1999; Hayes *et al.*, 2001; Philips and Griffiths, 1990; Rohde, 1985; Stewart, 1978). Being a highly nutritious medium, therefore many bacteria including spoilage and pathogenic bacteria can grow and propagate in it. Mastitis, milk quality and the presence of food-borne pathogens in unpasteurized raw milk either directly or indirectly increases the risk of ingestion and transmission of food-borne pathogens and ingestion of potentially harmful toxins. Many microorganisms can get access to milk and products, among these are *E. coli*, *Salmonella*, coliform, *Staphylococcus aureus*, yeast and mould and other bacteria.

Main objectives:

- 1- To evaluate the microbial quality of different cow milk samples

Specific Objectives:

- 1- To determine the total bacteria viable count of the milk.
- 2- To determine the total count of moulds and yeasts of the milk.
- 3- To detect the present of *Salmonella*

CHAPTER TWO

LITERATURE REVIEW

2.1. Definition of Milk:

Milk is defined as the normal secretion excluding colostrums obtained by normal milking methods from the lactating mammary gland of a healthy cow (Johnson, 1980). Milk was defined as the normal secretion of the mammary glands of mammal (Eckles and Macy, 2004).

Milk is the physiological secretion of the mammary gland of mammals and provides nourishment for their young. Throughout history man has recognized the milk value and dairy products as food not only for the young but also for the adults (Nickerson, 1999). Milk is an easy digestible, less cost, available and rich food. It is considered as the most economical complete food compared to other source (IDF, 1983).

2.2. Nutritive value of milk:

Milk is an excellent source of high biological value protein because it contains varying amounts of all essential amino acids that human body cannot synthesize and in proportions, resembling amino acids requirements (Miller, 2000). Milk cows provide significant amount of protein and most micronutrients; including calcium, B-group vitamins, (particularly riboflavin and B12, also thiamin, niacin and B6), vitamin A, Iodine, Magnesium, phosphorus, potassium and Zinc (Anita, 2001).

Although, the salts of milk are quantitatively minor constituents, they are of major significance to its technological properties specially to the stability and properties of the milk's protein system (Fox and Mc Weeny, 1998). Calcium from dairy products has greater bio-availability than calcium from vegetables (Bordy, 1999). The components of milk color are due to milk's

natural pigment concentrations; from carotenoide, protein and riboflavin (Anita, 2001)..

Milk lipid is considered to be one of the outstanding milk constituents with respect to presence of lipid classes, variety and number of identified fatty acids (Jensen *et al*, 1991). Milk lipids include anti carcinogenic compounds such as conjugated linoleic acid, sphingomyelin and butyric acid (Parodi, 1999).

Lactose (B-galactosyl -glucose) is the carbohydrate source in milk of almost all mammals. It was probably the first periodic compound the nature developed in order to support the healthy growth of infants (Harju, 1991). Lactose cannot be absorbed unless being hydrolyzed to monosaccharide's; glucose and galactose by intestinal lactase, which declines early in life to the point of virtual absence in adulthood, making them lactose intolerant (Vesa, 1999). In populations with an old dairying tradition, lactose malabsorption is rarer (Harju, 1991). The nutritional value of milk as a whole is greater than the value of its individual nutrients because of its unique nutritional balance (Wattiaux, 2000).

Milk is ancient as mankind itself, as the substance created to feed the mammalian infant. All species of mammals from man to whales produce milk for this purpose (Douglas, 1995). Milk is the single most complete food known to exist naturally (Tull, 1996). The nutrients in milk are readily digestible form, and little is wasted during digestion. Milk is a valuable food not only for babies, but for people of all ages. The most important in milk to human are protein, calcium, potassium, phosphorus, other trace element, vitamin A, riboflavin, thiamine and other B vitamin. Also milk is a fairly low – calorie food so it is a relatively expensive source of energy (Chamberlain, 1990). the nutritional value of milk for infants is very clear as it is usually the chief source complete protein, the value of milk protein depends primarily on their content of some essential amino acids that cannot synthesized by the body . Moreover, cow and human milk contain all the essential amino acids, which are readily absorbed

(O'Connor, 1995). He also mentioned that the high levels of calcium and phosphorus in milk important for bone and tooth formation in young children and these elements play a significant role in preventing osteoporosis in elderly people.

2.3. Importance of milk:

Milk is normal product of mammary gland secretion; it is a complex nutritious product that contains more than 100 substances that are either in solution, suspension or emulsion in water (Wattiaux, 2000). People in Africa use milk from cows, sheep, goats and camels, and of these source cow milk is the most widely produced and processed (FAO, 1990). The Ministry of Animal Resource (Wattiaux, 2000). gave an estimate of annual milk production of 7.88 million tons; 5 million tons cow milk, 1.9 million tons goat milk, 0.65 million tons sheep milk and camels only 0.33 million tons.

In addition, Milk is the only food that provides a well balanced array of essential nutrients including protein, fat, carbohydrates, vitamin and minerals in the form which is palatable, digestible and sanitary (Kordylas, 1990).

2.4. Types of Milk:

The types of milk include whole cow, liquid skimmed, condensed whole, condensed skimmed, evaporated and human (Tull, 1996).

2.4.1. Whole milk:

Most fluid milk is consumed in the form of pasteurized, homogenized, vitamin D fortified whole milk

2.4.2. Low fat milk:

Per capita consumption of low fat and skim milk has increased substantially over the past decade.

2.4.3. Skim milk:

Obtained after all or most of the milk fat removed from the whole milk.

2.5. Composition of Milk:

FAO (1997) reported that the composition of milk varies considerably depending on species, breast feeding, health status and stage of lactation the major constituents are water, fat, protein, lactose and mineral matter.

Table: Typical composition of cow milk

Fat%	Protein%	Lactose%	Ash%	Total solid%
3.9	3.2	4.6	0.72	12.6

Source: Harding and Ditton (1995)

2.5.1. Total solid (TS)

The total solid (TS) content of cow's milk in a dairy herd varied slightly from one season of the year to another ranging from 13.72 to 14.83 % (Khalifa and Bayoumi, 1966). (Idris *et al*, 1975) reported comparable values of TS to those reported by Khalifa and Bayoumi (1966). On the other hand, Khalid and higher values of total solids TS) content of cow's milk varying from 12.13 to 15.39%(Idris *et al*, 1975) .

2.5.2. Ash:

The ash of milk contains Potassium, sodium, calcium, magnesium, chlorine, phosphorus and sulfur in relatively large amount. Beside other small amount of Iron, Copper, Zink, Aluminum, Manganese, Cobalt and Iodine and traces of Silicon, Boron, Titanium, Vanadium, Rubidium, Lithium Strontium. The percentage of ash in the composition of milk is about 0.7% (Eckles and Macy, 2004). The ash content varies from 0.5 -0.72% (Khalid and Joseph,

1976). The ash figures below 0.75% may be due to the presence of add water (Pearson, 1976).

2.5.3. Moisture:

Johauson (1980) found that the water content to be 87.20%. Other average about 87 pounds in each 100 pounds milk is water. This water is not different from ordinary water and serves to hold in solution the soluble constituent of milk. The percentage of water varies from 89.c% although occasionally, an individual sample of authentic milk may exceed these limits. Any variation in the amount of other constituents is also reflected upon the water percentage (Eckles and Macy, 2004).

2.5.4. Protein:

The protein of milk great importance to human nutrition and it influences the behavior and properties of dairy products (Jenness, 1988). Philip (1984) reported that fluid milk contains approximately 3.5% protein, 80% of which is casein the remainders are whey proteins (globulin and albumin). The casein and whey proteins effective complement each other to give milk its high biological value. Proteins are among the complex of organic substances that contain carbon, hydrogen, oxygen, nitrogen, sulfur and sometimes phosphorus. The protein contains between 2.80 and 4.00 percent (Eckles and Macy, 2004). Johanson, (1980) found that the protein content to be 3.5%. Milk protein consists mainly of casein with few other protein fractions such as lactolbumin and lactoglobulin. It is an excellent source of proteins that contains of all essential amino acids required by human (Payne, 1990).

2.5.5. Lactose:

Lactose in cow`s milk ranges from 4.83_ 4.90 % (Ali, 1973). While Idris *et al.* (1975) claimed different values of lactose ranging from 3.40 to 6%

Khalifa and Bayoumi (1966) reported similar values. Philip (1984) reported that milk contains 4.8% lactose (the predominant carbohydrate). FAO (1997) reported that limit variation of lactose is 3.6 -5.5. Lactose accounts for about 54% of solids _not _fat of milk and contributes about 300% of the calories of the whole milk. Eckles and Macy (2004) reported that lactose is found only in milk. It's is reducing disaccharide which, upon hydrolysis , yields one molecule of galactose and one olecule of glucose and it has the formula $C_{12}H_{22}O_{11}$ and prolonged heating of aqueous solutions of lactose at temperature form 212 to 266 F (100 -130C) . Results in a decomposition which is indicated by a light brown or "caramel" color. Eckles and Macy (2004) reported that lactose has an important relation to the manufacture of the milk products, due to the fact that it is easily decomposed by bacteria Ahmed (2004) reported that lactose in the milk of Khartoum state which ranges from 4.32 to 4.289. Milk carbohydrates are sugars which are especially important for infant feeding because they prevent intestinal putrefaction by encouraging growth of acid – producing bacteria in the stomach. Sugar also affects the absorption of minerals such as calcium and phosphorus (Payne, 1990).

2.5.6. Fat:

The milk fat is most valuable constituent of milk; it is the food value of the milk (Eckles and Macy, 2004). It was found that the fat content average to give 3.8%. Fat gives milk its characteristics of smoothness. Flavor a color and it contains around sixty six different fatty acids emulsified and dispersed in water in small globules each globule being surround by membrane to prevent fusion (Chamberlain, 1990). The milk fats contain high proportion of short – chain fatty acids especially butaric acids, and enzyme such as phosphates and lipases that effect the flavor of milk (Kordylas, 1991). The fat content of milk is often used as guide to the quality of the milk, and may affect its price (Tull, 1996).

2.6. Physical properties of milk:

2.6.1. Appearance:

The opacity of milk due to its content of suspended particles of fat, protein and certain minerals. The color varies from white to yellow according to the coloration (carotene content) of fat, (Dairy Processing hand book, 1995). According to FAO (1986) abnormal appearance include pink color which results from pollution with blood, yellowish creamy may be due to colostrums large clots or flakes are found in sour milk or milk obtained from mastitis cow, visible dirt is indication of unhygienic production methods. In addition skim milk is more transparent with slightly bluish tinge.

2.6.2. Density:

The density of milk normally varies between 1.028- 1.034g/cm³ depending on the composition. (Dairy Processing hand book, 1995). Also from cow to cow and in different seasons of the year when water is added to milk the density decreases (IPC, 1999).

2.6.3. Flavor:

The flavor of normal whole milk is pleasantly sweet flavor. Off flavor may be obtained from exposure to light, (APHA, 1990).

According to IPC (1999) if the cow eats strong smelling fodder within two hours before milking, the smell and the taste may develop in the milk. Milk stored near strong smelling substances will absorb the flavor of these products.

Undesirable flavor of milk (Doreen and Page, 2001; FAO (1986) includes:

- Barny flavor: unpleasant distinct odor associated with poor ventilation of cow stable.
- Bitter or rancid flavor: bitter taste may occur in milk due to possibility of some physiological disturbance or may result from the activity of enzyme lipase and thus will be encountered with rancidities Bitterness associated with rancidity as accompanied by a strong Disagreeable odor.

- Cooked flavor: specially may be noted by the off smell. This can be demonstrated by sampling and testing milk which has been heated to various temperatures up to boiling point.
- Cowy flavor: result from Ketosis or acetone.
- Oxidized flavor: oily or sunshine flavor resulting from the oxidation of some fatty constituent of milk. It is characterized by quick taste reaction when the sample is taken to the mouth.
- Sour milk is due to milk of lactic acid producing organisms.
- Salty flavor: due to colostrums.
- Flat flavor: dilution of milk with water.

2.6.4. Sediments:

Manure, straw, feed, hair, insects and other extraneous matter which enter milk through careless handling and improper cleaning before milking, use of unclean equipment and cans(American Public Health Association, 1990).

2.6.5. Freezing point:

The freezing point of milk is the only reliable parameter to check the adulteration with water, (Kaplan, 1963; England and Neff, 1963).

The freezing point of milk from individual cows has been found to vary from -0.54_ 0.59C, (peter, 1959).

The internal osmotic pressures also define the difference in freezing point between the solution and the solvent (water) so that the freezing point depression is the measure of this osmotic pressure. When the composition of milk is affected due to physiological or pathological causes such as late lactation and mastitis is termed abnormal milk but the osmotic pressure and hence the freezing point remains constant tetra pack processing hand book; (Harding, 1999).

2.6.6. Acidity:

Normal milk is slightly acidic with pH falling between 6.5-6.7 with 6.6 the most usual value.

Titrateable acidity of milk is the amount of hydroxyl ion (OH⁻) of a given strength needed to increase the pH of a given amount of milk to pH of about 8.4, the pH at which the normal used indicator phenolphthalein changes color from colorless to pink, the test is done to find out how much alkali is needed to change the pH from 6.6 to 8.4.

In Sudan Ibrahim (1973), reported that the mean titrable acidity was 0.18 +0.06 for vendor and 0.20 for dairy farm.

Idris *et al.* (1975) found that the mean Titrateable acidity for the Sudanese cattle milk was in range of 0.16- 0.226 with mean of 19%. Mohammed (1988) found that titrable acidity of raw milk in Khartoum North was 0.18-0.21 Khartoum 0.17-0.20.

2.7. Dairy microbiology:

Milk is a sterile at secretion in udder but is contaminated by bacteria even before it leaves the udder. Except in the case of mastitis, the bacteria at this point are harmless and few in number. Further infection of milk by microorganisms can take place during milking, handling, storage and other pre-processing activities.

Lactic acid bacteria: this group of bacteria is able to ferment lactose to lactic acid. They are normally present in milk and are also used as starter culture in the production of cultured dairy products such as yogurt.

2.7.1. Bacteria count in milk:

Due to very specific composition, milk is susceptible to contamination by a wide variety of bacteria (Kotnis).

Farm milk may contain anything from a few thousand bacteria per ml if it comes from hygienic farm up to several millions if the standard of cleaning and handling is poor, high atmospheric temp, poor cooling and transport,

(Kurweil and Busse, 1973). The bacterial count the cfu (Colony Forming Units) should be less than 100,000 cfu per ml (Dairy Processing hand book, 1995).

The total bacterial count (TBC) is intended to indicate the level of micro-organisms in the product APHA, (1985).

The total bacterial count is used to indicate the overall bacterial quality of milk [FAO, WHO, (1992)] and to study the hygienic and sanitary condition under which milk are produced, handled, transported and processed (Clupei and Hill, 1967).

Both temperature and storage time influence the multiplication of bacteria, the storage temp is significant for the type of bacteria growing time and spoilage effect on the milk, (Kerstin, 2000).

Damaged teats can affect milk quality in that any break in the skin can become a reservoir for mastitis bacteria and give rise to a significant increase in bacterial count, Harding, (1999).

2.7.2. Microbial growth:

There are a number of factors that affect the survival and growth of microorganism in food. The parameters that is inherent to the food, or intrinsic factors, including the following:

- Nutrient content.
- Moisture content.
- PH.
- Available oxygen.
- Biological structures.
- Antimicrobial constituents.

Nutrient requirements: While the nutrient requirements are quite organism specific, the microorganisms of importance in foods require the following:

- Water.
- Energy source.

- Carbon/nitrogen source.
- Vitamins.
- Minerals

Milk and dairy products are generally very rich in nutrients that provide an ideal growth environment for many microorganisms.

Moisture content: All microorganisms require water but the amount necessary for growth varies between species. The amount of water available in food is expressed in terms of water activity (a_w), where the a_w of pure water is 1.0. Each microorganism has a maximum, optimum and minimum a_w for growth and survival. Generally bacteria dominate in foods with high a_w (minimum approximately 0.90 a_w) while yeasts and moulds that require less moisture, dominate in low a_w foods.

2.7.3. Micro- organisms in milk:

2.7.3.1. Spoilage microorganisms in milk:

The microbial quality in raw milk crucial for the production of quality dairy food. Spoilage is a term used to describe the deterioration of a foods texture, color, odor or flavor to the point where it is unappetizing or unsuitable for human consumption. Microbial spoilage of food often involves the degradation of protein, carbohydrates and fats by the microorganisms or their enzymes.

In milk, the microorganisms principally involved in spoilage are psychotropic organisms, most psychrophiles are destroyed by pasteurization temperatures, and however, some like *Pseudomonas fluorescens* and *Pseudomonas fragile* can produce proteolytic and lipolytic extracellular enzymes which are heat stable and capable of causing spoilage. Some species and strains of *Bacillus*, *Clostridium*, *Corynebacterium*, *Arthrobacter*, *Lactobacillus*, *Mycobacterium*, *Micrococcus* and *Streptococcus* can survive pasteurization and grow at refrigeration temperatures which can cause spoilage problems.

2.7.3.2. Pathogenic microorganisms in milk:

Hygienic milk production practices, proper handling and storage of milk and mandatory pasteurization have decreased the threat of milk borne diseases such as tuberculosis, brucellosis and typhoid fever. There have been a number of food borne illnesses resulting from the ingestion of raw milk, or dairy products made with milk that was not properly pasteurized or was poorly handled causing post-processing contamination. The following bacterial pathogens are still of concern today in raw milk and other dairy products:

- *Bacillus cereus*.
- *Listeria monocytogenes*.
- *Yersinia enterocolitica*.
- *Salmonella spp.*
- *Escherichia coli*.
- *Campylobacter jejuns*

2.7.4. Bacterial species present in raw milk:

2.7.4.1. Gram positive bacteria in milk:

2.7.4.1.1. The genus *Staphylococcus*:

Usually found in cluster, the most important effect is their ability to produce enters toxins which cause food poisoning. According to their reaction with coagulase test, they are divided into coagulase positive (CPS) which includes pathogenic staphylococci and coagulase negative (CNS) which includes the pathogenic and non pathogenic species of genus *Staphylococcus*, (Devriese 1999).

2.7.4.1.1. *Staphylococcus aureus*:

Produce number of extracellular compounds including haemolysis, staphylococcal enter toxins, coagulase, nuclease and lipase. 10% of staphylococci causing mastitis in cows produce a heat stable enterotoxin and may cause food toxicity to man I.D.F, (1980).

In Sudan many workers isolated *S. aureus* strain from bovine milk and showed that they were the most frequently isolated udder pathogens (waken and Eltayeb, 1962; Ibrahim and Hubbell, Adlan, *et al*, 1980).

Staphylococcus aureus is considered the world's third most important cause of food-borne illnesses. The ability of *S. aureus* to grow and produce SEs under a wide range of conditions is evident from the variety of foods implicated in staphylococcal food poisoning. Indeed, milk is a good substrate for *S. aureus* growth and dairy products are known sources of food-borne illness. *S. aureus* contamination can occur through the presence of *S. aureus* in the raw milk itself or during its processing (1-5). Cows are probably the main sources of contamination of raw milk with enterotoxigenic *S. aureus* strains. In particular, cows with subclinical *S. aureus* mastitis can release large quantities of *S. aureus* into their milk (6). Although pasteurization kills *S. aureus* cells, thermostable SEs generally retain their biological activity (7). Thus, because of the importance of these toxins in the public health and food sectors, an efficient screening method to detect the prevalence of enterotoxic strains in foods is required. Indeed, not all staphylococci produce SE and SE production may be insufficient for the contamination of food products. Traditionally, 7 classic antigenic SE types have been recognized: SEA, SEB, SEC1, SEC2, SEC3, The detection of classical enterotoxins of *Staphylococcus aureus* in raw cow milk using the ELISA method320 SED, and SEE. During the 1990s, new SEs (SEG,SEH, SEI, and SEJ) were reported and their genes were described. More recent data resulting from partial or complete genome sequence analyses have led to the description of further “new” SE genes: SEK, SEL, SEM, SEN, SEO, SEP, SEQ, SER, and SEU. The role of these new SEs in food poisoning has not yet been clarified (8-11). SEA is the most common enterotoxin recovered From food-poisoning outbreaks in the US (77.8% of all outbreaks) followed by SED (37.5%) and SEB (10%) (12). the intoxication is generally not lethal although the elderly are more susceptible to mortality from food borne-induced

gastroenteritis than younger individuals (7). Surveys to detect classical enterotoxins and identify enterotoxin genes in *S. aureus* from milk and milk products have been conducted in many countries including Italy (8), Norway (3), Turkey (9), and Brazil (10). Investigations should also be performed to find the relationship between the presence of this pathogen or SEs in food and the ability to cause disease in humans, as was outlined by (Ali and Abdelgadir, 2009)

2.7.4.2. Gram negative organisms:

2.7.4.2.1. Coliform bacteria:

Coliform are Gram negative rod-like, lactose fermenting bacteria natural inhabitant of colonic flora which spread via faecal contamination to in environment. Coliform can spread from one udder to another (Kaper *et al.*, 2004). Devriese, (1999) stated that coliform presence in milk of high numbers may be due to improper handling which allow the bacteria multiplication.

2.7.4.2.2. Detection of coliform bacteria in milk:

In addition to the use of solid media, liquid media which can be worked out by the most probable number (MPN) is used (APHA, 1985). MPN method has been shown to produce satisfactory results with naturally- contaminated foods for the detection of *coliforms*, faecal coliform, and Coliform and *E. coli* are often used 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 *et al.*, 2004). The output of dairy and dairy products from Sudan is increasing day by day in their internal market. Considering its economic potential, extensive and intensive exploitation of cow milk can both contribute to the nutrient requirements of the

Sudanese public and increase the income of farmers. In view of the growing public awareness about food safety and quality, knowledge of the microbial and chemical composition of milk is of great significance for further development of its hygienic processing into high quality consumer products. Until now, information on such aspects is scant and scattered. Thus, the objective of this study was to investigate the occurrence of the opportunistic pathogen *E. coli* in cow's milk in Khartoum State, Sudan. As was outlined by (Ali *et al*, 2011).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Materials:

Raw milk samples were collected from different locations of Khartoum North (markets, Farms, street Vendors) it has been kept in refrigerator to be used later on. All chemicals used in this study were of analytical grade and obtained from Khartoum University laboratories.

3.2. Methods

3.2.1. Microbiological analysis:

3.2.1.1. Media used:

- 1-Plate count Agar
- 2-Nutrient Broth
- 3-Pootato Dextrose Agar
- 4-Macconkey Broth
- 5-Brilliant Green 2% Bile Broth
- 6-EC Broth
- 7-Eosin Methylene Blue Agar
- 8-Selenite Cystine Broth
- 9-Bismuth Sulphit Agar
- 10-Baird-Parker Agar

3.2.1.2. Diluents used:

- 0, 1% Peptone solution

3.2.1.3. Sterilization of glassware:

Petri dishes, test tubes, flasks, Pipettes, ECT were sterilized in hot air oven at 160-180 for 2 to 3 hours. Before they were washed, deried and packed in stainless steel cans or sometimes in aluminum foil.

3.2.1.4. Sterilization of glassware:

Culture media were first adjusted to the required PH and then sterilized. Sterilization was achieved by autoclaving at 121c for 15 minutes.

3.2.1.5. Total viable count of bacteria:

It was carried out by using the Pour plate count method as described by W.F Harrigan (1998). Suitable medium for this purpose is plate count Agar.

3.2.1.6. Preparation of serial dilution:

Aseptically 10 grams of the sample were homogenized in 90 ml of sterile diluents (0, 1% peptone solution). It was mixed well to give dilution (10^{-1}).

By using sterile pipette 1 ml was transferred aseptically from dilution containing 9 ml of sterile diluents and it was mixed well to give dilution (10^{-2}).

In the same way the preparation of serial dilution was continued until the dilution (10^{-6}).

One ml of each dilution was transferred into sterile Petri dish to each plate 15 ml of sterile melted plate count agar were added .The inoculums was mixed with medium and allowed to solidify.

The plates were incubated at 37c for 48 hours.

A colony counter was used to count the viable bacterial colonies after incubation and the results were expressed as colony forming unit [cfu] per gram



Plate 6: Peptomterter

3.2.1.7. Determination of Coliform bacteria:

It was carried out by using the most probable Number (MPN) technique. Presumptive *Coliforms* test 1 ml of each of the three first dilutions (10^{-1} , 10^{-2} , 10^{-3}) was inoculated in triplication of macConkey broth tubes containing Durham tubes. The tubes were incubated at 37 c for 48 hours. The production of acid with sufficient gas to fill the concave of the Durham tube is recorded as positive presumptive test.

Confirmed test for Total Coliform

From every tube showing positive result a tube of Brilliant Green 2% Bile Broth was inoculated by using sterile loop. The tubes were incubated at 37 c for 48 hours. Then the tubes showing positive and negative result were record. The

most probable number of total coliform was found out by using the most probable number (MPN) tubes.

3.2.1.8. Confirmed *E.coli* test:

Medium used EC Broth. From every tube showing positive result in the presumptive test a tube of EC broth containing Durham tube was inoculated. Then the tubes were incubated at 44.5 c for 24 hours. Tubes showing any amount of gas were considered positive. The most probable number (MPN) was record. For further confirmation of *E.coli*. From every tube of EC broth showing positive result at 44.5 c for 24 hours a plate of E.M.B agar [Eosin Methylene Blue agar] was inoculated by using sterile loop .The plates were incubatet at 37 c for 48 hours. Colonies of *E.coli* are usually small with metallic green sheen on EMB agar.

3.2.1.9. *Staphylococcus aureus* enumeration:

Medium used was Baird-parker medium. About 0, 1 ml of every dilution was transferred onto the surface of each well dried Baird-parker medium plate using sterile font glass rod. The plates were incubated at 37 c for 24 hours. After the period of incubation had been finished the plates were examined.

Staphylococcus aureus after 24 hours appear black shiny convex and surround by ozone of clearing 2-5 mm in width of colony.

3.2.1.10. Yeast and Moulds:

From suitable dilution of sample 0.1 ml was aseptically transferred onto solidified potato Agar containing 0.1 g cholraphenicol per one liter of medium to inhibit bacterial growth .The sample was spread all over the plates using sterile bent glass rod. Plates were then incubated at 28 c for 72 hours. Colonies were counted by using a colony counter and the result were presented as CFU/gram.

3.2.1.11. Detection of *Salmonella*:

10 gram of the sample were added to a conical flask containing 100 ml sterile Nutrient Broth and incubated at 37 c for 24 hours. Aloopful of 24 hours incubated nutrient broth was transferred aseptically into sterilized selenite cystine broth and incubated at 37 c for 24 hours. Aloopful of 24 hours inoculums of selenite cystine broth was streak on Bismuth sulphite agar surface and incubated at 37 c for 24 – 72 hours. Black metallic sheen discrete colonies indicated the presence of *salmonella*.

3.2.2. Statistical analysis:

The results were subjected to Statistical Analysis System (SAS) by using One-Factor Analysis of Variance (ANOVA). The Mean values were also tested and separated by using Duncan's Multiple Range Test (DMRT) as described by Steel *et al.* (1997).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1. Microbiological analysis of milk samples:

4.1.1. Determination of Total viable count (TVC):

Table (1) shows that TVC of bacteria in the milk samples taken from farms is 3.65 cfu/ml, taken from groceries is 4.78 cfu/ml and from milk vendors is 6.47 cfu/ml. The highest contamination (6.47cfu/ml) in the sample taken from milk vendors may be due to lack of cleanliness of the surrounding environment, or exposure milk to contamination by open utensils. The lowest TVC was observed in the sample taken from Farm. These results were in disagreement with those reported by Ali (2013) who stated that the TVC of milk of Khartoum North is 9.88cfu/ml. These differences may be due to feeding tripe, farming practice hygiene etc.

4.1.2. Determination of yeasts and moulds:

Table (2) shows that yeasts and moulds in the milk samples taken from farms is 0.897 cfu/ml, taken from groceries is 0.94cfu/ml and from milk vendors is 2.95cfu/ml. The highest contamination in the samples taken from milk vendors may be due to lack of hygienic practices. The lowest contamination was observed in sample taken from farms may be due to good practices for milking on the farm, These results in were disagreement with those mentioned by Ali (2013) who stated that the yeasts and moulds of milk of Khartoum North is 9.63cfu/ml these differences may be due to the milking environment or pots and milk etc.

4.1.3. Determination of *staphylococcus aureus*:

Table (3) shows that *staphylococcus aureus* in the milk samples taken from farms is 1.18cfu/ml, taken from groceries is 2.05cfu/ml and from milk vendors is 3.40cfu/ml. The highest contamination in the sample taken from milk vendors may be due to mishandling and milk exposed to dust and dirt pots. The lowest contamination was observed in sample taken from farms may be due to good practices for milking on the farm. These results were in a good agreement with those reported by Ali (2013) who stated that the *staphylococcus aureus* of milk of Khartoum North is 1.2cfu/ml, this result agreed with us in farms sample but differed in the rest of the results.

4.1.4. Determination of Coliform:

Table (4) shows that the Total Coliform in the milk samples taken from farms is 5.11MPN/ml, taken from groceries is 18.57MPN/ml and from milk vendors is 49.1MPN/ml. The highest contamination in the sample taken from milk vendors may be due to contamination of milk with faecal, The lowest contamination was observed in sample taken from farms may be due to less contamination with fecal. These results in were disagreement with those reported by Ali (2013) who stated that the Total Coliforms of milk of Khartoum North is 5.43MPN/ml this result agreed with us in farms sample but differed in the rest of the results.

4.1.5. Determination of *E. coli*:

Table (5) shows that the *E. coli* in the milk samples taken from farms is 1.11 MPN/ml, taken from groceries is 1.89 MPN/ml and from milk vendors is 15.0 MPN/ml. The highest contamination in the sample taken from milk vendors may be due to infected of cow's with Mastitis and lack of hygienic practices. The lowest contamination was observed in sample taken from farms may be due to less contamination in milk. These results in were disagreement with

those mentioned by Ali and Abdelgadir (2011) who stated that the *E. coli* of milk of Khartoum North is 3.93MPN/ml these differences may be due to farming practice etc.

4.1.6. Determination of *Salmonella*:

Table (6) shows that *Salmonella* was not detected in the modern farm, while detected in traditional farm and not detected in the supermarkets, while detected in traditional shop and all milk vendors. This result in agreement with Ali (2013) *Salmonella* was detected in milk collected from Khartoum North.

Table (1): Total viable count of bacteria ($\log_{10}$ cfu/L) of milk collected from sources in Khartoum State

Source of milk	TVCB
Farms	3.653 ^b ±0.03
Groceries	4.787 ^{ab} ±0.92
Milk vendors	6.470 ^a ±0.97
Lsd _{0.05}	2.482 [*]
SE±	0.7172

Values are mean±SD.

Mean(s) bearing different superscript(s) in columns and rows are significantly different ($P \leq 0.05$) according to DMRT.

Table (2): Yeasts and moulds ($\log_{10}$ cfu/L) of milk collected from sources in Khartoum State

Source of milk	Yeasts and Moulds
Farms	0.897 ^b ±0.03
Groceries	0.940 ^b ±0.63
Milk vendors	2.950 ^a ±0.97
Lsd _{0.05}	2.166 [*]
SE±	0.7685

Values are mean±SD.

Mean(s) bearing different superscript(s) in columns and rows are significantly different ($P \leq 0.05$) according to DMRT.

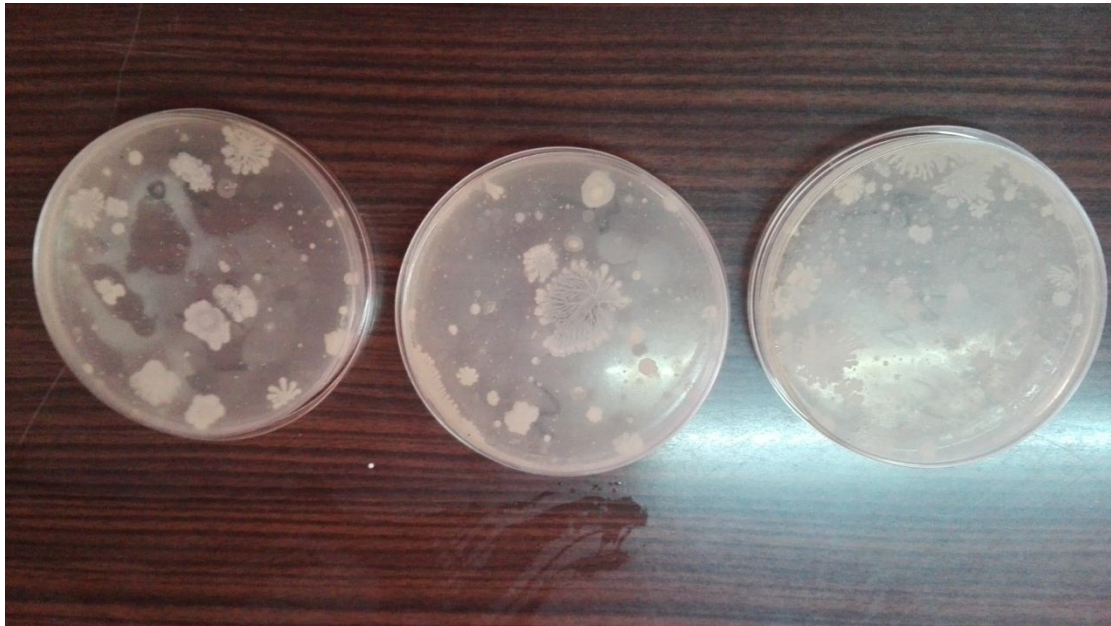


Plate 7: Total viable count of bacteria

Table (3): *Staphylococcus aureus* (log₁₀ cfu/L) of milk collected from sources in Khartoum State

Source of milk	<i>Staphylococcus aureus</i>
Farms	1.187 ^c ±0.26
Groceries	2.050 ^b ±0.84
Milk vendors	3.407 ^a ±0.73
Lsd_{0.05}	1.293 [*]
SE±	0.9517

Values are mean±SD.

Mean(s) bearing different superscript(s) in columns and rows are significantly different (P≤0.05) according to DMRT.

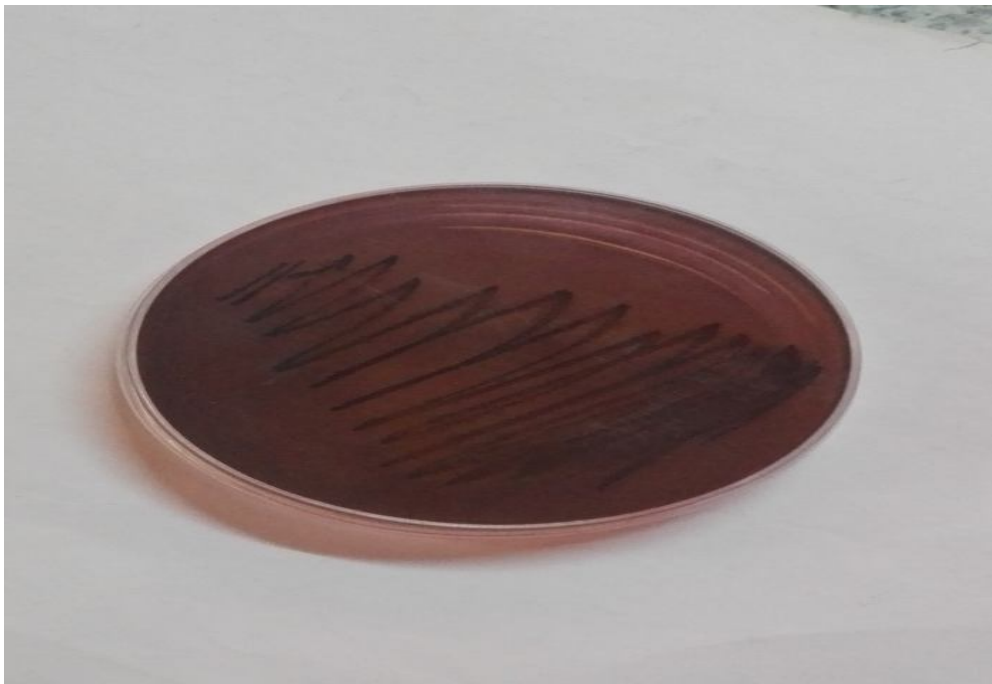


Plate 8: *Staphylococcus aureus*

Table (4): Total coliforms (MPN/L) of milk collected from sources in Khartoum State

Source of milk	Total coliforms
Farms	5.11 ^c ±0.60
Groceries	18.57 ^b ±0.97
Milk vendors	49.10 ^a ±13.41
Lsd _{0.05}	8.57 ^{**}
SE±	1.225

Values are mean±SD.

Mean(s) bearing different superscript(s) in columns and rows are significantly different (P≤0.05) according to DMRT.

Table (5): *E. coli* (MPN/L) of milk collected from sources in Khartoum State

Source of milk	<i>E. coli</i>
Farms	1.110 ^b ±0.19
Groceries	1.89 ^b ±0.27
Milk vendors	15.00 ^a ±3.41
Lsd_{0.05}	5.581 ^{**}
SE±	1.613

Values are mean±SD.

Mean(s) bearing different superscript(s) in columns and rows are significantly different (P≤0.05) according to DMRT

Table (6): Detection of *Salmonella* of milk collected from sources in Khartoum State

Sample	Farms	Groceries	Milk vendors
Sample (1)	Sudan university	supermarket	Box ₁
	-	-	+
	-	-	-
Sample (2)	-	-	+
Sample (3)			
Sample (1)	Khartoum university	Semi supermarket	Box ₂
	-	-	+
	-	-	+
Sample (2)	-	-	-
Sample (3)			
Sample (1)	Traditional	Traditional shop	Donkey
	+	-	+
	-	+	+
Sample (2)	+	+	+
Sample (3)			



Plate 9: Total coliforms and *E. coli*



Plate 10: *Salmonella*

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATION

5.1. Conclusions:

From the obtained results we can conclude for the following:

- The total bacteria count was found in high number in the vendor's milk, while the lowest number was found in the farms and groceries.
- A gain the total moulds and yeasts was found in high number in the vendor's milk, while the lowest number was found in the farms and groceries.
- The pathogenic microorganism was also determined in high number in the vendor's milk, while determined in lowest number in the farms and groceries.

5.2. Recommendations:

- People must buy milk from clean places such as supermarket stores and not from milk vendor.
- From this study routine follow up and checking of farms animals should be a available
- We must sell milk in containers clean and free so as not attached microbe's vessels.
- Good manufacture practices are recommended.
- The responsible person should apply the lows against those who mishandled the milk production.
- Further studies are recommended.

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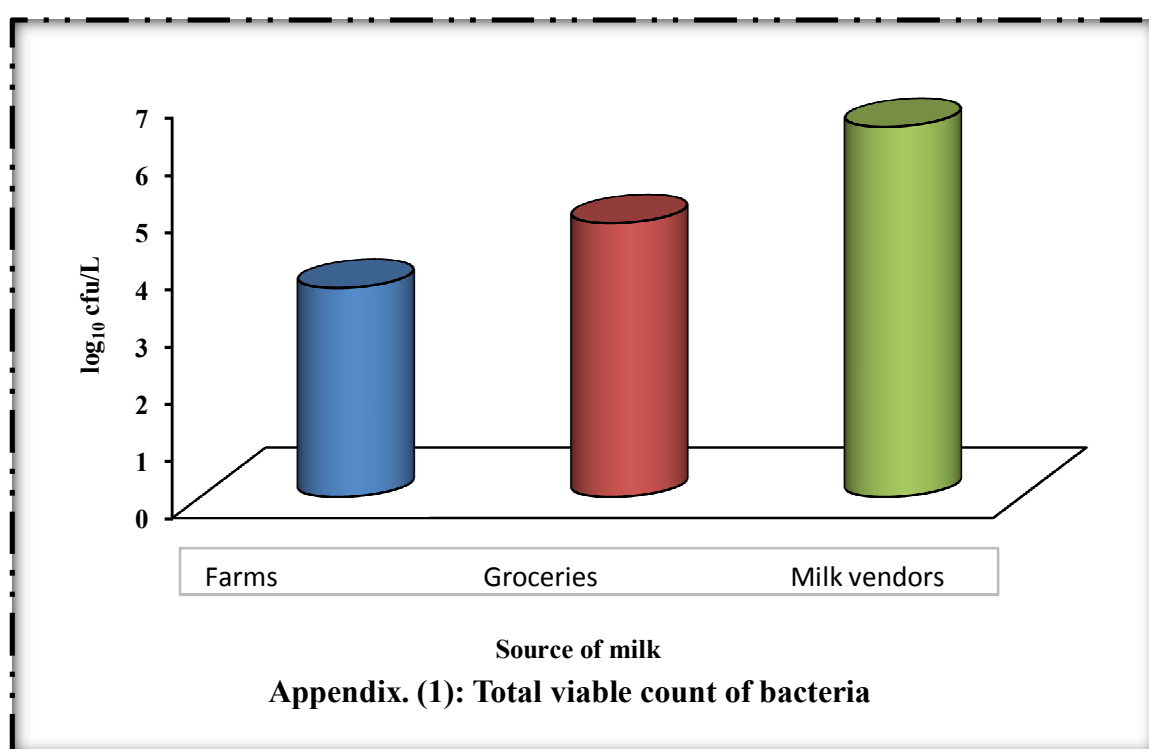
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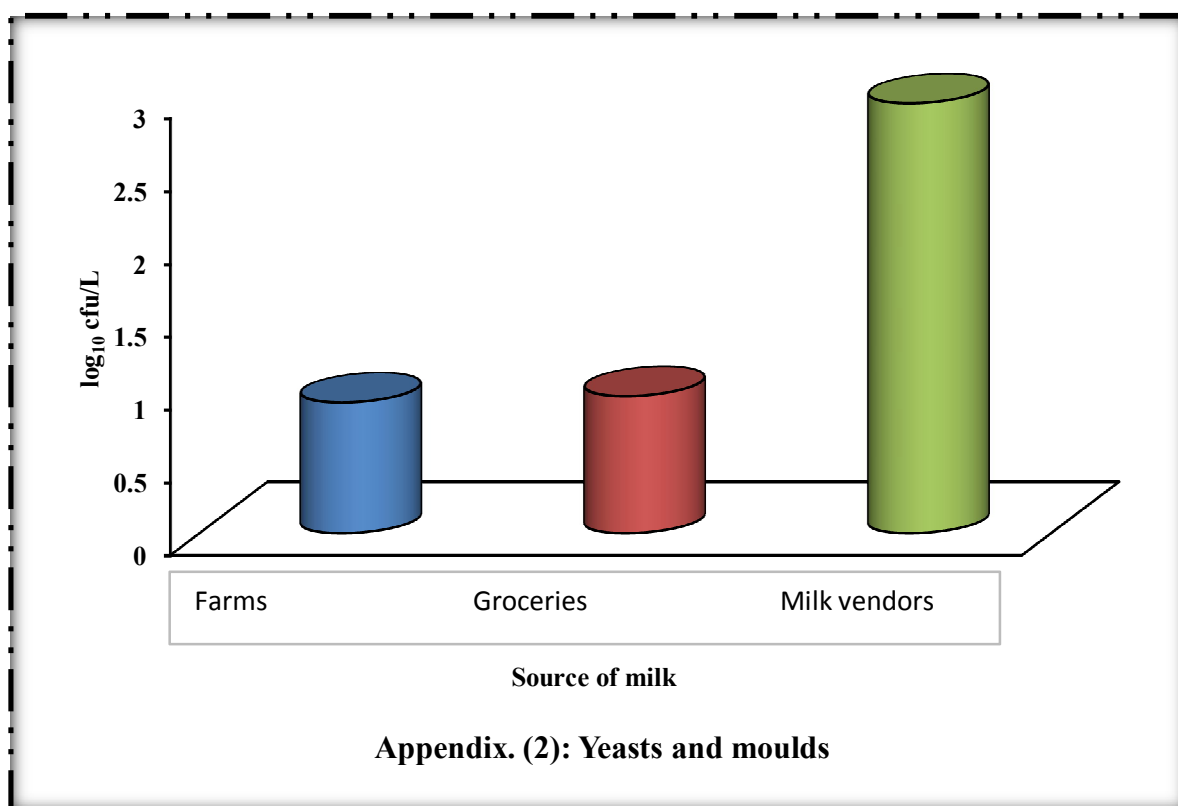
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Appendices





Appendix. (2): Yeasts and moulds

