MICROBIOLOGY AND CHEMICAL COMPOSITION OF FERMENTED COW URINE (OKAH)

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DEDICATION

To my:

Parents,
Sisters & brothers

Prof. Hamid Ahmed Dirar

And all my friends
ACKNOWLEDGEMENTS

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ABSTRACT

This study was conducted to isolate and identify the microorganisms of Okah (fermented cow urine) and to determine its chemical composition also. Okah samples were collected from the Nuba Mountains villages where this food is produced. Routine microbiological methods were used to isolate, enumerate and identify the microorganisms.

The study revealed that the microbial load of Okah ranged from $3.1 \times 10^2$ to $6.8 \times 10^5$ under aerobic conditions and from $3.1 \times 10^2$ to $3.5 \times 10^4$ under anaerobic conditions. The prevailing bacteria in the collected samples were *Bacillus coagulans*, *Bacillus licheniformis*, *Bacillus brevis*, *Bacillus macerans*, *Staphylococcus* spp., *Micrococcus* spp. and *corynebacteria* spp. Beside these bacteria some yeast were also found.

A number of bacteria species was isolated and identified during the laboratory fermentation of urine. These included *Bacillus*, *Corynebacterium*, *Staphylococcus*, *Enterococcus* and *Lactobacillus*.

The chemical composition of Okah samples revealed that it consists of 92.95% moisture, 2.98% protein, 0.25% fat and 3.82% ash. The minerals content of the samples was 1.66, 0.11, 1.21, 0.16, 4.91, 1.45, 2.05 and 3.3 for potassium, sodium, nitrogen, phosphorus, calcium, magnesium, zinc and ferrous, respectively.

Similar results were obtained with Okah prepared in the laboratory. It has 92.3% moisture, 0.01% fat, 7.2% protein and 1.03% ash.

The study showed that Okah contained more protein than urine.
أ.جلود: 1،6-أكسيكسيك-2-أكسيكسيك-أكسجين

المادة Chúng: البُكْرَة

البحث: هذه الدراسة تهدف إلى تحسين ومراقبة تأثيرات الأوكسجين الدقيق على الأحياء والتدخلات السريعة في الأوكسجين إلى الأعضاء.

النتائج: تُظهر النتائج أن الأوكسجين الدقيق يؤدي إلى زيادة في عدد البكتيريا في الأ🏆نة. كما أن الأوكسجين الدقيق يُحسن من القدرة على تكوين الأوكسجين في الأ🏆نة. 

المصطلحات: البُكْرَة، الأوكسجين، القدرة، تكوين الأوكسجين.

النوع: بُكْرَة
CHAPTER ONE
INTRODUCTION

Fermentation is the oldest form of food preservation technology in the world, indigenous fermented foods such as bread, cheese and wine have been prepared and consumed for thousands of years and strongly linked to culture and tradition, especially in rural households and village communities.

Fermented foods are popular through the world and in some regions make a significant contribution to the diet of millions of individuals (Okafor, 1992).

Moreover SteinKraus (1983) considered that the value of traditional fermented foods for human survival, development, welfare and importance in counteracting world hunger cannot be underestimated nor ignored. Their importance is increased enormously when such foods are considered important elements in the diet of millions of people, especially in the developing countries.

Unconventional products are used as food in many parts of the world. Africa, Far East and Mexico come first in consumption. Many unconventional products are used such as meat meal, lungs, gastrointestinal tract, tendons, hides, ligaments, horns, hair, wool, blood, Non Protein Nitrogen (NPN), Single Cell Protein (SCP) and insects. In Sudan the unconventional foods include cow urine, bone, locust, caterpillars, bile juice, frogs and such marginal foods as hooves and hides (Dirar, 1993; Cheeke, 2005).

The people of Diling of Nuba Mountains prefer to consume Okah made of a fermented cow urine. Okah has a strong odour and
salty taste. Okah is fermented cow urine used in this area as a food and medical treatment. Fermented Okah can be preserved for a long time which may reach ten years.

The main objectives of this work are summarized as follows:

1. Isolation and identification of microorganisms associated with fermentation of Okah.
2. Chemical composition of collected samples of Okah.
4. Study of the microbial and other changes during fermentation of Okah.
CHAPTER TWO
LITERATURE REVIEW

2.1 Fermentation

Fermentation is one of the oldest and most economical methods of producing, preserving, processing food and improving food safety. In addition to preservation, fermentation can also contribute to the improvement of nutritional value. During fermentation the unique properties of the bacteria and fungi present that increase the levels of proteins, vitamins, essential amino acids and fatty acids in the food. Some microorganisms produce flavouring compounds, complex polysaccharides or organic acids and development of desirable colour (Harlander, 1992).

At present, fermentation is used to mean the use of submerged liquid culture of selected strains of microorganisms, plant or animal cells, for the manufacture of some useful product or products (Neil and Harvey, 2008).

Most traditional fermented foods from natural fermentation are carried out under non-sterile conditions. The environment resulting from the chemical composition of the raw materials, fermentation temperature, absence or presence of oxygen, and additives such as salt and spices causes a gradual selection of microorganisms responsible for the desired product characteristics (Nout, 1992).

Fermented foods are defined as desirable processes of biochemical modification of primary food products brought about by microorganisms and their enzymes (Steinkraus, 1996).
Fermentation is purposely carried out to enhance properties such as taste, aroma, shelf-life, texture and nutritional value. Also often part of sequence of food processing operations, including cleaning, grinding, soaking, salting, cooking, packaging and distribution (FAO/WHO 1996; López, 1992).

All fermented foods have aroma and flavour characteristics that result directly or indirectly from the fermenting organisms (Laskin, 1982; Steinkraus, 1995; Jay, 1986).

**2.1.1 The benefits of fermentation**

Fermentations have developed in many parts of the World as a low cost method for food preservation for months and years. It is important in developing countries where preservation techniques such as cold storage or hot-holding are not used due to lack of facilities and resource, canning, refrigeration and freezing not a viable. Fermentation usually requires little energy and improves the nutritional value of foods by bio-enrichment with microbial protein, amino acids, lipids and vitamins (Cook, 1991; Dirar, 1992b).

Fermentation processes can also contribute to the degradation of toxins and anti nutritional factors present in many plants. Furthermore, the organoleptic properties of fermented foods often differ from the unfermented substrate; in addition to a better flavour than raw product and aroma, the physical form, improved texture and colour may change (Cook, 1991; Dirar, 1992b).

Fermentations methods are inexpensive and destroy undesirable factors in the raw product, the process involves little waste (López, 1992; Dirar, 1992a).
Fermentations has been considered as an affordable technology for the safe preservation of foods, in particular weaning foods. In developing countries, as a result of poor hygienic handling and inadequate preservation, weaning foods are often contaminated and are minted and are a major cause of diarrhea and associated malnutrition (FAO/WHO, 1996).

Lactic acid fermentation of preserved foods is characterized by a significant acidity caused by the production of lactic acid. Lactic acid fermentation was evaluated as a part of food preparation processes involving other operations such as soaking, cooking and the germination of cereal grains. The use of germinated cereals is of particular interest since they can be used to prepare semi-liquid porridges of high nutrient density. Many lactic acid bacteria have a better tolerance to salt than most pathogenic bacteria. Salt is also effective in protecting the product against some spoilage bacteria (Motarjemi and Nout, 1996; Steinkraus, 1995).

Fermentation produces change in nutrient composition. Carbohydrates, particularly starch and soluble sugars, are the principal energy source of fermenting microorganism. The level of these compounds as well as non-digestible oligosaccharides decrease during the microbial fermentation. Certain amino acids may be synthesized during fermentation (Svanberg and Lorri, 1995).

Fermentation results in an increase in the B complex vitamins (folic acid and vitamin B_{12}) especially, riboflavin in cereals and legumes and vitamin K. Probiotics also produce short-chain fatty acids, an important energy source for colon bacteria. Some amino acids of proteins hydrolysis, growth factors, antioxidants and anti
microbial factors are also produced. Also it destroys certain anti
nutrients phytates and oxalates that interfere with the absorption of
minerals like iron and calcium (Svanberg and Lorri, 1995; Dirar,
1996).

Fermentation is used as a technique in the purification of
certain components of the raw material (starch). Also good health and
long life are generally linked to fermented milks, in particular to
yoghurt and have the ability to produce antibiotics, the process
reduces the toxicity of some food (for example, Gari and Penjeum),
while others may become extremely toxic during fermentation
(Bongkrek) (Dirar, 1992a; Jay, 1986).

Preservation of fermented food in substantial amounts through
lactic acid, alcohol, acetic acid and alkaline results in decrease in
cooking time and fuel requirements.

Fermented foods may help to protect the consumers against
diseases by providing minute doses of antibiotics produced by the
microorganisms effecting the fermentation.

2.1.2 Fermented foods in the world

Fermentation is a common traditional and economical house-
hold technology in many parts of the world (FAO/WHO, 1996).

The history of fermented foods in the world has early record
in Southeast Asia, where china is regarded as the cradle of mold-
fermented foods, Egyptians and Sumerians both had knowledge of the
techniques used to convert starchy grains into alcohol (Nout, 1992;
Neil and Harvey, 2008).
In many parts of the world, Africa, Latin America, Far East, South Asia and south East Asia fermented foods form an important diet and nutrition of the poor and economically deprived (Aidoo, 1992; Harlander, 1992).

Fermentation of cereal grains is used to prepare a variety of foods. Fermented products from maize are usually found in Africa, central and south America (guinea corn) and millet in Africa and south Asia. Foods based on rice are practiced in India, China, South East Asia and the Far East. Foods from wheat are important in Middle East, Turkey and the Far East (Aidoo, 1992).

Bread has been produced by man for thousands of years and is one of the few foods common to many societies. The history of bread is known early in Egypt and it is eaten almost everywhere. Those cereals are available and are now being consumed more commonly in regions, such as in South East Asia (Jenson, 1997; Wood, 1989).

Bread, in its most simple form, uses only flour, yeast, salt and water as ingredients. Most bread is produced with wheat flour, but it may also be produced with rye or corn flour. Cereals form the base of numerous beverages such as Aliha in South Africa and Tesguino (a fermented maize beverage).

In many countries where cows, goats and sheep have been domesticated there is a number of interesting fermented foods based on milk. In the world, there are over 1200 cheeses prepared from milk. Cheese originated in the Middle East. It is a rich source of minerals calcium and phosphorus, fat (20 to 30%) and a range of vitamins. The classification of cheese depend upon the texture or moisture content (hard-cheddar, semi hard-Gouda and soft). About Fourteen percent of
cheese is produced by the European union. In France there are 370 varieties of cheese. Sixty percent of cheddar is produced in UK and in Holland 97% is gouda (Farr, 1982; Stanley, 1997; Sozzi, 1980).

Yoghourt, fermented milk produced with starter, is found in most areas in the world such as North Africa, Zimbabwe; Egyptian Kishk also found in Syria, Jordan, Lebanon and Iraq, Greek Trahanas also found in the eastern and Mediterranean and Middle East countries. Turkish Tarhans are mixtures of sheep's milk yoghurt and parboiled wheat. Koumiss is fermented milk produced by lactic acid and alcohol fermentation (Steinkraus, 1992).

Saké is the traditional alcoholic beverage of Japan. The raw materials are rice, water and inoculation starter is a mixture of the mould Aspergillus oryzae and the yeast saccharomyces (Fleet, 1997; Sozzi, 1980).

Cassava is an important food crop in the tropics and in many countries in Africa, South America and Mexico. Also, there are many fermented foods produced from cassava such as Gari (Oyewole, 1992; Farr, 1982).

Fermented fish products make large part of foods in some countries. In Thailand people make 16 fermented fish products (Sundhagul et al., 1975). In the Philippines fermented fish products can be divided into two groups. The first group, includes those containing high concentration of salt, about 15 to 20% in the final product. This group consists of Bagoong (fish paste) and patis (fish sauce). The second group includes, Burongisda which is a fermented rice fish mixture and Burong hipon also known as Balobalao which is a fermented shrimp rice mixture (Olymbia, 1992).
Soy bean is produced in most regions of the world, China, Indonesia, Japan, Korea, East and South East Asia and United States. It is a source of high protein and oil. In Nigeria, soy bean is used to make Dawadawa. Dawadawa is also known as Iru or Ogiri. In the conventional method of brewing soy sauce, cooked soy beans and roasted wheat are mixed with spores of *Aspergillus spp* and fermented in solid culture for 2 days to produce Koji. The Koji is then mixed with brine to make Moromi (Yokotsuka and Sasaki, 1997).

Fresh hip muscle of pork meat give a better quality fermented pork (Nham), a popular dish in Thailand. It consists of fresh pork meat that is trimmed, minced, mixed thoroughly with salt and rice and wrapped in small banana leaf packets. Fermented sausages give fermented meat in Western countries such as pepperoni and salami (Jay, 1986). Some people in United States who were for a long time vegetarian started to change their habit and eat fermented oriental products made from fermented soy bean past called Miso (IFS, 1985, Farr, 1982).

### 2.1.3 Fermented foods in Africa

If it is true that Africa is the origin of man, as most archaeological and genetic findings suggest and since it has been established that tool making hominids were present in tropical Africa two million years before their appearance in Europe and Asia (Dirar, 1993), then the first man to consume a fermented food must have lived in Africa, probably East Africa. Dirar(1993) reported that the food fermentations have even pre-human origin.
Fermented foods of Africa be found with the Africa woman because, African women who naturally made indigenous African foods from indigenous African raw materials, animals and plants.

Fermented foods have early records in Africa where the Egyptians developed the concept of the combined brewery-bakery. The early Egyptian beers were probably quite similar to some of the traditional opaque sorghum, maize, or millet beers found in various African countries (Nout, 1992).

In Africa, there are found diverse kinds of fermented food products; cereal grains account for more than 60 percent of food materials used in the preparation of indigenous fermented foods (Aidoo, 1992).

In savannah fermented products from legumes and other seeds are important food condiments and are generally strong smelling (Aidoo, 1992).

Odunfa (1985, 1988) reported that Africa has over 30 different fermented foods, and that one country such as Nigeria has over 20 (Odunfa, 1981). The Sudan alone, however contains 80 fermented foods and beverages that are distinctly different from each other (Dirar, 1993).

Kuboye (1985) noticed that most of the traditional Nigerian staple foods and soup condiments are fermented products. The acidic porridges play an important role in the daily diet. Two African words given to similar fermented dough type or there products are Uji of Kenya and Ogi of Nigeria (Mbugua, 1984 Akingbala et al, 1981). Dawadawa (fermented soybean) is the most important food flavouring
condiment in West Africa. A part from its flavouring attributes, it is used in poor rural families as low cost meat substitute (Odunfa, 1986).

African fermented foods can be broadly grouped into three functional groups: the staples, the relishes and sauces and the beverages (Dirar, 1996).

2.1.4 Fermented foods in Sudan

In the Sudan, women have invented some 90 different fermented foods, many of which are well documented to be at least two thousand years old. There are foods for quenching thirst, foods for replenishing blood sugar, food for providing quick energy, portable foods for travelers, foods for new mothers, foods for wedding occasions and food for the fasting Muslim (Dirar, 1993).

The Sudan contains many different fermented foods. The diversity of these foods stems from the diversity of raw starting materials from which they are made. The raw materials to be fermented include the better known products such as sorghum, millet, milk, fish and meat. Also, a number of unorthodox raw materials are fermented such as bones, hides, skins, hooves, gall bladder, fat, intestine, caterpillars, locusts, frogs and cow urine (Dirar, 1993).

Many fermented foods and drinks are made from sorghum malt like Jiriya, Khemiss Tweria and Merissa beer, which are found in 12 different bread types prepared from sorghum, its quite surprising such as Abreh and Kissra, these foods contain high content of protein.

There are some eleven types of fermented meat products. The most important is a member of jerky types. Strips of meat are either sun dried or shade-dried and fermented to give the product. The other
fermented products are a bit unorthodox like Birta and Miriss. Birta and Miriss are fermented caul fat. Dodery is a fermented fresh cow bones made from some kinds of bone called germasha (fragile bone).

Fermented fish in Sudan are greater than other regions of Africa. Kejeik (large sun-dried split fish); Fessikh (salted fermented whole tiger fish); Mindeshi (pounded small fish paste, fermented, and may be dried later); and Terkin or meluha (fermented fish sauce or paste-not dried).

Fermented milk products are common in Sudan. Rob is milk fermented overnight. The milk is churned to give butter (furssa), which is later boiled to give butter-oil. Gariss (fermented camel milk), Biruni or leben-gedim (fermented unchurned milk ripened for up 10 years); Jibna beida (white cheese), zabadi (yoghurt), and black cumin-flavored mish, are the fermented dairy products (Dirar, 1992a).

A number of different fermented plants products were also found. These include Kawal (fermented green leaves of wild legume plant); frundu (fermented seeds of *Hibiscus sabdariffa*); Sigda (fermented sesame seed cake), all contain protein rich in sulphur amino acids (Dirar, 1992; Harper and Collins, 1992).

There are some fermented unconventional products like Okah (fermented cow urine); Beiga (fermented caterpillars); Duga (fermented locusts); and Kesherneh (fermented frogs) (Dirar, 1993).

2.1.5 Fermented foods in Nuba Mountains

Most fermented foods of the Sudan (about 80%) are to be found west of the Nile, in the states of Kordofan and Darfur. The reasons are unclear, but perhaps these have been spreading from an origin in the
west, far or near, and the great river has acted as formidable physical barrier to their effective spread eastward.

Nuba mountains are situated in southern Kordofan. Many tribes lived in these areas (approximately 99 tribes). The tribes have very simple life, therefore seem to ferment anything like Doud-maneh, Okah and sigda (or um-afona a fermented sesame paste prepared to simulate the strong flavour) to live. In the Nuba mountains Merissa is a most important food for many tribes, such as those of Korongo, Kadugli, Miri, Buram, Tira and Akhdar mountains. Kanyu-Moro in the Nuba mountains is an alcoholic drink made from fermented oil seeds by Moro tribe, the Nuba of southern Kordofan, Jebel Fenda, Jebel Kujuriya, Jebel Kerbu, Jebel Yima consume a fermented meat product made from lungs, kidney, liver, spleem, heart and the caul fat are all chopped and mixed into a pot with milk to produce Beirta. Within the western Sudan itself, two groups of mountain dwelling communities seem to possess more knowledge of fermentation than other groups. These are the Fur of Darfur and the Nuba of the mountains, both considered indigenous people (Dirar, 1993).

2.1.6 Flavours and substitutes

The USA Food and Drug Administration (FDA) defines flavouring agent and adjuvant as substances added to impart or help to impart a taste or aroma in food. FDA identifies flavour enhancer as substance added to supplement, enhance, or modify a characteristic taste or aroma of its own.

Natural flavour or natural flavouring means the essential oil, oleoresin, essence or extractive, protein hydrolysate, distillate or any product of roasting, heating or enzymolysis, which contains the
flavoring constituents derived from a spice, fruit or fruit juice, vegetable or vegetable juice, edible yeast, herb, bark, bud, root, leaf or similar plant material, meat, seafood, poultry, eggs, dairy products, or fermentation product thereof, enzymatic microbiological process from material of vegetable or animal origin either in the raw state or after processing for human consumption by traditional food-preparation processes including drying, to rarefaction and fermentation (Burdock and Fenaroli, 2004).

The fermentation takes a photolytic course, giving a foul-smelling product, the flavour of which simulates, after cooking, that of fermented meat and sour milk.

The flavours and substitutes to be divided into two groups. The first group is of plant origin and the second group is made from animal origin.

Flavours and substitutes made from plant origin are used to provide those of meat and sour milk. Meat flavours and substitute such as Kawal, Sigda, Furundu, Amilinguwi, Kerjigl, Lawass and Mushrooms. These foods are high quality protein and rich in sulfur amino acids (Dirar, 1984; Dirar., 1985; Odunfa, 1986, Elfaki, 1991; Elfaki, 1992).

Sour milk substitutes include rob-ful (peanut butter-milk) and rob-heb (from water melon seeds). The minor sour milk substitutes include um-zummata and moyat-aish. These products are analogous to soy yoghurt and peanut butter milk (Andres, 1978). They are all made from oil seeds.
Another flavour and substitutes made from animal origin such as Okah, Beiga, Duga and Itaga (Dirar, 1992b).

2.2 Unconventional Products

These are made from raw materials that don’t enter into the main stream of what is normally considered food; these include Okah, Beiga, Duga and Kesherneh (Dirar, 1992b).

The most common nonprotein nitrogen (NPN) source is urea. It is produced in the liver from ammonia arising from amino acid metabolism in animals. Urea is secreted from liver into the blood. It is used in animal feeding and fertilization. Urea excreted in the urine contains 45 percent nitrogen; it contains 281 percent protein (N×6.25). Other sources of urea are human and animal urine. In India urine is used as treatment of human disease and by Nuba tribes as a cure for malaria and snake bites in Sudan (Cheeke, 2005; Dirar, 1993).

In rumen, microbial enzymes degrade dietary protein to amino acids and then ferment the amino acids as energy sources, excreting the nitrogen as ammonia.

Biuret is another source of NPN produced by heating urea to a high temperature, resulting in the condensation of two urea molecules.

Meat meal derived from slaughter house wastes and plant products contains liver and inedible offal (lungs, gastrointestinal tract, tendons, hides, ligaments, horns, hair, wool and blood). If bone is included, it is known as meat and bone meal. Meat production results in substantial quantities of non-meat materials such as bone and fat, which must be converted into by-products by rendering processes. Batch dry rendering is generally used where the material contains
bone, giving tallow and dry meat and bone meal. In the Sudan bone is used for food in Darfur such as Dodery and Kaidu-digla.

Blood is used in the diet of animals. Blood now is collected to be used directly for human food. It contains approximately 80% crude protein high in lysine.

The protein forages (alfalfa) has a good amino acid balance for nonruminant animals and humans.

Single Cell Protein (SCP) refers to bacteria, yeast, algae and fungi. Algae used as human food, have a high concentration of protein and are excellent source of xanthophyl and carotene pigments. Chlorella and Spirulina (cynobacteria ) are used as food in Chad and Mexico. Healthy food contains 65-70% protein.

Insects have been used as food sources by humans in some parts of the world. Men eat grass hoppers, beetles, crickets, caterpillars, the pupae of butter flies and moths, termites, ants and bee larvae (Cheeke, 2005; Herbert and Kearney, 1975; Desrosier, 1970).

Fish used as diets for poultry and young animals are of high quantity and quality protein. Also a good source of fish oil is used for human nutrition (Hulan et al., 1988, 1989).

2.3 Urine of some domestic animals

Urine is blood filtered by the kidneys and the resulting filtrate undergoes a series of modification within the renal tubules. (Aspinall and Oreilloy, 2004).

Urine is not a waste product, but a purified, sterile watery solution, it is an extraordinary valuable physiological substance. It is composed of many nontoxic substances, the toxic one’s are being
removed from the body through the liver, intestine, through the skin, and through out breath (Yagil, 1985 and 1992).

The composition of urine depends on the life style of humans, and on the type of grazing pasture of animals. Natural urine contains a measurable amount of substances which has been used medically, even in extremely large quantities without causing side effects (Elbeshir, 2004).

The urine is very different in composition and volume from the original filtrate. For every 100L of fluid filtered from the blood only 1L is produced as urine. Ninety nine percent of the original filtrate is reabsorbed back into the blood.

Normal urine contains only water, salts and urea, and doesn't contain glucose. Normal urine of most animals is clear, watery fluid usually yellowish in colour (Aspinall and Oreilly, 2004).

In horses it is viscous due to presence of mucoprotein secreted from the kidney and in chickens is creamy and thick due to presence of uric acid, which is excreted with faeces from the cloacal (Ibrahim, 1989).

The pH of urine in herbivorous animal is alkaline and in carnivores is acidic and this is due to type of food they eat.

Body fluid and excretions such as urine, saliva and faeces can change in colour, volume, frequency of excretion, consistency and smell, which often points to specific disorder.

The odour of camel urine is unpleasant but it changes in Trypanosoma evansi infected camel. In this case the odour changes to acacia-flower-like odour (Hussein and Gunid, 1993) or fruity-aroma-
like-odour (Kleiner and Orten, 1962) and this is due to presence of large amount of acetone in infected camel urine.

Camel ability to adapt to extreme aridity of the habitat is unique amongst large herbivores. Camel can take a very large amount of water compared to other animals. This would result in severe osmotic problems. Camels do this because water is absorbed very solely from their stomach and intestine. Furthermore, their erythrocytes can swell to 240% of normal size without bursting (other animal can only go to 150%). Water losses through urine are minimized by concentrating urine, by reducing renal urine flow and by retaining metabolites in the body fluids. Camel daily output of urine ranges between 0.5-5 liter/day depending to a large extent on the animals status of hydration or dehydration (Elkhalifa, 2003; Schmidt-Nielsen, 1964).

Drinking saline water increases the urinary volume and electrolyte excretion in sheep and cattle relative to those drinking fresh water (Eltayep, 2006).

2.3.1 Cow Urine Definition and uses

Urine is water which contains soluble waste products. It is filtered from blood during the passage through the kidney. It contains waste products concentrated in the blood and must be lowered. So kidney can reabsorb substances which are needed to maintain the constant composition of blood (Ibrahim, 1989).

Cow urine is cow’s blood that is filtered by kidney whatever elements are present in blood are present in cow urine also.
Urine is usually yellow or clear depending on health and diet, and certain foods can affect the odour. The colour may change to red or black, due to copper or iron in the urine.

Cow urine is consumed in Sahel from southern and western Sudan to the Atlantic Coast and in small pockets of East Africa such as Kenya (Dirar, 1993).

Cow urine is used in many parts of Africa as a source of salt which is very hard to find (Bloch, 1963).

Muslims do not drink fresh urine, since urine, in general, is regarded from a religious point of view, as filth, compelling ablution before prayer (Dirar, 1993).

2.3.2 The components of cow urine

Cow urine contains nitrogen (N, NH2), Sulphur (S), Ammonia (NH3), Copper (Cu), iron (Fe), Urea [Co (NH2)2], Uric acid (C5H4N4O3), phosphate (P), Sodium (Na), Potassium (K), Manganese (Mn), Carbolic acid (HCOOH), Calcium (Ca), Salt (NaCl), Vitamins (A, B, C, D, E). Other minerals, Enzyme, lactose, water H2O, Hipuric acid, creatinin C4HgN2O2, creatin and Aurum hydroxide (Jain, 2002).

Urine contains many other waste products in minute quantities. The months in which cow gives milk, the cow urine contains lactose which is very beneficial for heart and brain disorders and salt (Jain, 2000).

Cow urine contains vital nutrients, vitamins, hormones, enzymes and critical antibodies.
Many useful elements have been found in urine such as urea. Urea is a major element found in urine and is the end product of protein metabolism, a principle end product of protein catabolism in mammals and much of it is excreted through the kidneys. It is highly soluble, relatively compound and a reasonably high proportion of urea produced in the liver. Most may be degraded to ammonia by bacteria (Frappe, 1986).

Urea is secreted from the liver into the blood, filtered out in kidney and excreted in the urine.

Urea is the most common NPN source. It is a normal product of metabolism. It is produced in the liver from ammonia arising from amino acids metabolism in animals.

Urea is utilized by being converted to ammonia in the rumen by the action of microbial urease. Urea is used in animal feeding and fertilizer is produced chemically. It is non-toxic but can cause ammonia toxicity (Cheeke, 2005).

Uric acid is similar to urea and has strong anti bacterial properties, it is mono anion urate, is traditionally considered to be a metabolically inert end-product of purine (Becker, 1993).

Creatine is found in the urine of subjects ingesting creatine monohydrate as an organic aid. The catabolic break down product of creatine is a major constituent of normal urine (Darren et al, 1998).

Minerals from urine can be very easily reabsorbed as compared to those derived from food. Urine probably contains more different types of minerals than those derived from food.
Urine becomes turbid if left alone for a while. This is because of the presence of enzymes. In urine, dissolve urea change it into ammonia then urine becomes strongly alkaline making it difficult to dissolve rich minerals.

Bioactive substances and hormones such as urokinase, epithelium growth factor, stimulating factor, growth hormone different bioactive effective effects such as promotion of protein production. Cartilage growth, fat decomposition, erythropoetine, gonadotropins, tripsyn and allantoine (Jain, 2002).

2.3.3 Urine therapy

Different types of medicines, with plant, animals or even earth origin, have been discovered and tried throughout history. Some of them are no longer used while others have been modified and developed along with the development of technology.

Historically, the medical usage of urine was quite well known throughout the world. There are many reports that date back thousands of years which extol the virtues of urine both as a diagnostic tool and as a medical treatment for a wide range of diseases, wounds, and skin disorders. Urine as a medicine is used externally or internally to promote or to maintain mammalian health. This is based on the principle of nature cycle. Urine therapy has proven helpful in a great number of various diseases ranging from simple cold and throatache, to tuberculosis and asthma (Kroon, 1996). Also in minor skin problems such as itching to more serious skin disease such as eczema, psoriasis, and even skin cancer. Urine therapy can be combined with any other natural medicine to yield good results. Nowadays cancer is treated in different parts of the world by urine therapy(Elbashir,2004).
Urine therapy has been practiced and known in eastern and western countries for thousands of years, especially within Yoga and Tantra tradition where use of urine has been kept alive. The way of the Yoga is the practice of ingesting one’s own urine in Rome, India and Egypt (Burzynsky, 1986). German encyclopedia stated that the Greek and Romans were acquainted with the use of urine as a medicine (Plesch, 1947). The Englishman who was the urine therapy pioneer, cured himself of tuberculosis, which had been declared “incurable” (Elbashir, 2004). Also an Australian scientist found a hormone in morning urine called melanine.

2.3.4 Cow urine therapy in the world

Cow urine therapy is practiced in many parts of the world such as Japan, India, Nigeria and Sudan. Fermented cow’s urine is used as medicine in different cultures (Awale et al, 2006).

Myanmar uses cow urine for therapy over 2500 years. Since Buddhist era, people in this area believe that cow urine medicine is the most powerful medicine that can cure any kind of ailment. Medicine is made from cow’s urine fermented with three different kinds of medicinal fruits in different proportions. Cows urine is pickled with three medicinal fruits: *Phyllanthus emblica*, *Phyllanthus simplex* and *Terminilia chebula* and fermented in a clay pot in the ratio 1:1:2. The cow’s urine is kept into container above the level of the fruits and left for at least 3 months. People believed it can cure all kinds of diseases including cancer and it is used as a blind therapy. Certain symptoms may arise after drinking such that the body may feel warm immediately, or they may experience palpitation, dizziness, vomiting, frequent urination and diarrhea.
Budha recommend the Haritaki (*Terminalia chebura*) impregnated with cows urine for a monk suffering from jaundice (Awale *et al*., 2006).

In India, Vedas are source of culture. As kamdhenu is provider of all happiness, the presidents of India drink a glass of fresh cow urine daily early in the morning, for good health. The urine of the cow at any age can be used for treatment. Three types of cow urine treatments are used, fermented called Gaumutrasar (fermented cow urine with jaggery), distilled cow urine called Gaumutra Arka and tablet called gaumutra ghanvatic (Dirar, 1993, Jain, 2000).

Cow’s urine has been described as universal medicine for improving general health in Ayurveda. Cows urine is one of the ingredient of pancha gavya ghrity, an ayurvedic formulation prepared with five components of cow origin, viz cow milk, ghee, urine, dung and curd in equal proportions and is claimed to be useful against liver disorder (Awale *et al*., 2006).

Cow’s Urine Concoction (CUC) has been used for many years among the Yoruba's in the south west of Nigeria as remedy for convulsion in children. CUC is a popular traditional remedy for convulsive seizures in Nigeria. Its major pharmacological actions include anticonvulsant and hypoglycemic effect (Awale *et al*., 2006, Oyebola, 1983).

CUC is prepared from medium size leaves of tobacco (*Nicotiana tabaccum*), garlic, basil, lemon juice, rock salt and bulbs of onion. These are soaked in the cow’s urine when the active principles in these constituents dissolve. Over fifty chemical compounds have been identified in CUC. The major compounds it contains are benzoic
acid, phenyl acetic acid, p-cresol, thymol and nicotine. Observations of CUC poisoning in man and experimental animals showed that the main effects of CUC are severe depression of respiration, cardiovascular system, the central nervous system and hypoglycemia. These toxic effects were found to cause death (Jimoh et al., 2000; Oyebola and Elegbe, 1993; Oyebola, 1983).

In Sudan, fresh cow urine is widely used in parts of southern Darfur and Kordofan as medicine against malaria. The Nuba use a cow urine for bathing and drink part of the liquid as treatment for malaria and measles. The Dinka of upper Nile commonly drink fresh cow urine as a substitute for water to quench their thirst when the water is not available. They also wash the utensil for milk by urine and wash the hair and hand. Washing hair with urine, the hair became red in colour and that means this man own more cows (Dirar, 1993; Elkhalifa, 2003; Ohaj, 1998).

In southern Sudan people drink cow urine for curing fever. Fermented cow urine is also boiled with tamarind and red pepper and the resulting soup is drunk together with laben-gedim as a cure for malaria and snakebites (Dirar, 1992b). People use to drink Okah as medicine become healthy after drinking but some symptoms may arise these include vomiting.

Ibn Sina, and Ibn Elbitar (undated) reported that ox-urine was used to treat ear infection and cow urine used to cure ulcer.

Cow urine inhibited the growth of *E. coli* only on C.L.E.D. agar (O’haj, 1993). It has a germicidal power to kill a variety of germs, also destroys poisons, gives strength to brain and heart and protects brain and heart from damages.
Cow’s urine medicine is also a popular Buddhist treatment among the Buddhist people in Thailand and Sirlanka (Awale et al., 2006).

2.3.5 Other animals urine treatment (camel, goat)

Camel’s urine therapy, particularly urine of one-humped camel (*Camelus dromedarius*) is medically used for centuries in different parts of Arab countries. It is mostly used for chronic problems and it has been recommended by Prophet Mohammed (peace and blessings of Allah be upon him) for treatment of some diseases. Prophet Mohamed (peace be upon him) recommended camel’s urine and milk as treatment for people who have pale faces and abdominal distention (Elbukhary, undated). (Some sick people came to Prophet Mohammed (peace be upon him) and they were pale-faced and had abdominal distention. They asked for treatment and the prophet (peace and blessings of Allah be upon him) advised them to use camel’s urine and milk (Elbukhary, undated). Also Prophet Mohammed (peace and blessings of Allah be upon him) advised people with abdominal disorder to use camel’s urine (Elbukhary).

Ancient Arabs used to boil camel urine to treat their patients in Yemen. They used to dry it under the sun and press it in shape of tablets. These tablets were used for treatment of bad burns and wet bloody injuries (Ibn-Elbitar, undated). Also Ibn sina mentioned the use of camel urine in treatment of ascitis and achieved successful result Alrazi, printed, 1958) (O’haj, 1998).

Recently, camel urine has been used for treatment of Leukemia and digestive system cancer in Kuwait (O’haj, 1993).
Also it is being used as hair detergent (shampoo) in Morocco, some part of Sudan, Saudi Arabia and Somalia (Ibrahim, 1989; O’haj, 1993; Elkhalifa, 2003). O’haj 1998) mentioned the use of camel urine for treatment of fever or malaria.

In Arabian desert, people used to give the urine of young camel’s mixed with milk to overcome the bad odour and taste and every morning as treatment of cancer and leukemia cases (Kabariti, et al., 1988).

72% of nomadic tribes in eastern Sudan use camel urine for treatment of internal problems in general, while 52, 33, 20 and 33% used it for malaria, ascitis, dental problem and as hair shampoo, respectively (O’haj, 1993).

O’haj (1993) reported that the camel’s urine contains high levels of potassium, albuminous proteins and small amounts of uric acid, sodium and creatine.

Donkey urine was used for treatment of nephritis (Ibn sina, undated). Ibn Elbitar (undated) mentioned the use of a mixture of all animal urine for injuries and ulcers.

Goat urine was reported to be used for fever treatment (Ib sina, undated). Alrazi (printed, 1985) reported the use of goat urine mixed with medicinal plants for treatment of jaundice and ascites.

O’haj (1998) found that goat urine inhibited the in vitro growth of Staphylococcus aureus (120%), (100%) and (100%) on nutrient agar, MacConkey agar, C, L, E, D. Agar , respectively.
2.4 Fermented cow urine

2.4.1 Okah preparation method

The use of fresh cow’s urine for various purpose is quite common among various tribes of the Sudan.

In the Sudan and specifically in the Nuba Mountains, urine of cow is fermented and ripened for up to 10 years to be used as human food.

Around the town of Diling the product is called Okah, a name also given to fresh, unfermented urine. Other Nuba tribes call the fermented product tinurki twaril, while the Arab tribes who undoubtedly took the practice of urine fermentation from the Nuba, call the product bol-gedim (ripened urine). Both Arab and Nuba in southern Kordofan produce and consume Okah (Dirar, 1993).

Not all cow urine may be fermented; only a special kind that of young heifers. Moreover, only the urine produced during the rainy season.

Every morning the intended cow urine is collected and poured into a clean old zeer or burma, preferably one used for drinking water the earthenware container.

After the three months women take the container and incubate it for six months.

2.4.2 Okah mulah and sauce

Okah is used to prepare mullah for sorghum or millet aceda, using the usual ingredients such as okra powder, water, salt, etc. For a small family, half a teacup is sufficient to make sauce. Okah gives mullah a tea-red colour and unique flavour. Although the product is said to be used as a meat substitute.
Sometimes the intact green leaves of the cowpea plant are boiled in Okah, the juice sieved and weika (okra powder) added to it to give mullah kunjara. The leaves themselves are sun dried and stored to be used at convenience as a salad after mixing with sesame paste in the same way as for boiled kawal plant leaves.

2.4.3 Okah other names

The researcher conducted a personal survey (2007) to the Diling area in Nuba mountains in Southern Kordofan to collect samples of Okah for the purpose of the study. Okah is a fermented cow urine. The natives of this area prepared Okah for the purpose of curing malaria. It is also used by the Nuba tribes as a medicine for stomach diseases and in food as flavour. Many tribes made fermented cow urine (Okah) by different methods and named it by different names but the common constituent of Okah is cow urine.

The Yiming tribe called the fermented cow urine Aguida., Kujuriya tribe called it Tena orkha, Diling tribe call it Shondy and Fenda tribe call it Okah.

The common thing among all these products is cow urine. The fermentation of cow urine was carried out in the same way with slight differences in the methods used by the different tribes. Some tribes prefer heifer urine others use the urine of milking cows and cow after 40 days following parturition. Some tribes ferment urine alone others add other constituents such as bones (especially cow or goat bones containing fat), corn after peel and cowpea leaves.

Generally the urine is collected in the early morning or early night. Most people prefer to collect cow urine during the rainy season.
The urine is poured in old zeer or burma (Earthen pot or plastic pot) or bukhssa. Some people boil it for concentration. The containers are tightly covered and allowed to stay, the colour of urine changes to red or brown or black and the taste changes in addition to odour.
3.1 Materials

3.1.1 Collection of samples

Okah samples were collected from Diling homes, villages of Jebel Fenda and Shambat (Elmersa). Eight samples of Okah were collected in plastic containers delivered within 2 days and were stored at room temperature in the laboratory.

3.1.2 Sterilization of glassware:

Before sterilization, glassware were washed carefully and left to dry, then sterilized in a hot oven at 160°C for 3 hours (Harrigan and McCance, 1978).

3.1.3 Culture media used

3.1.3.1 Solid Media

3.1.3.1.1 Plate count agar (Oxoid) Modified

The medium was composed of tryptone, yeast extract, D-glucose and granulated agar. It was prepared according to manufacturer’s instructions by using 23.5g in 1 liter (fresh cow urine). The medium was allowed to boil in water bath until it was completely dissolved. The pH was adjusted to 7.0, then the medium was sterilized in an autoclave at 121 C° for 20 minutes. The medium was used for aerobic and anaerobic counting of microflora.

3.1.3.1.2 Nutrient agar (Oxoid) Modified

The components of the medium were peptone, yeast extract, lab-lemco powder, sodium chloride and agar. It was prepared according to the manufacturer’s directions by using 40g in one liter of
fresh cow urine and boiled in a water bath until it was completely dissolved. The pH was adjusted to 7.2 and autoclaved at 121°C for 15 minutes. It was used for sub-culturing of bacteria (Harrigan, 1998).

3.1.3.1.3 Malt extract agar (Oxoid) Modified

The medium was composed of malt extract 30.0g, mycological peptone 5.0g and 15.0g agar dissolved in one liter of fresh cow urine by boiling and then sterilized by autoclaving at 121°C for 15 minutes. To inhibit bacterial growth 100 mg of an antibiotic, chloramphenicol were added to one liter of the molten medium immediately before pouring the plates. The medium was used for enumeration and isolation of yeast and molds.

3.1.3.1.4 Staphylococcus 110 medium (Oxoid) Modified

The medium was composed of yeast extract, tryptone, lactose, mannitol, sodium chloride, dipotassium hydrogen phosphate, gelatin and agar. Exactly 150g were added to one liter fresh cow urine. The pH was 7.1. The medium was sterilized in autoclave at 121°C for 20 minutes. This is a selective medium used to detect and count *Staphylococcus*.

3.3.1.5 De man, Rogosa and Sharpe (MRS) agar Modified

The constituents of the medium were peptone, lab-lemco, meat extract, yeast extract, D-glucose, tween 80, Dipotassium hydrogen phosphate, sodium acetate, triammonium citrate, magnesium sulphate MgSO4. 7H2O, and manganese sulphate MnSO4. 4H2O. According to the manufacturer’s instructions, it was prepared by using 55g in one liter fresh cow urine. The medium was allowed to boil in water bath until it was completely dissolved. The pH was adjusted to 6.5 and the
medium autoclaved at 121ºC for 15 minutes. It was used to count and isolate lactic acid bacteria (LAB).

3.1.3.1.6 Starch hydrolysis medium

The medium consisted of nutrient agar with the addition of 0.2% soluble starch and sterilized at 121ºC for 15 min.

3.1.3.1.7 Blood agar

Nutrient agar medium containing 0.85% sodium chloride was sterilized and liquefied, cooled to 50 ºC and 5ml fresh sheep blood were added to 10 ml of nutrient agar in sterile Petri dish (Harrigan, 1998).

3.1.3.2 Semi-solid media

3.1.3.2.1 Hugh and Leifson’s medium (O/F)

The medium consisted of peptone, sodium chloride, dipotassium hydrogen phosphate, bromothymol blue 1% aqueous solution. The medium was sterilized in the autoclave at 121ºC for 15 min. The glucose was prepared separately from the basal medium as 10% solution and sterilized by autoclaving at 110ºC for 10 minutes, then added aseptically to the medium to give a concentration of 1%. The medium was used for differentiating oxidative and fermentative metabolism of carbohydrate (Harrigan, 1998).

3.1.3.2.2 Motility medium

The medium was composed of nutrient broth. Ten grams agar were dissolved in one liter distilled water and sterilized by autoclaving at 121ºC for 15 minutes after dispensing into test tubes (Steel, 1974).
3.1.3.3 Liquid Media

3.1.3.3.1 Glucose phosphate broth

The medium consisted of peptone, D-glucose, dipotassium hydrogen phosphate and distilled water (final pH 7.5) then distributed in 5 ml amounts in test tubes, sterilized by autoclaving at 115°C for 20 minutes and used in Voges-Proskauer test for identifying species of Bacillus.

3.1.3.3.2 Nitrate broth

The medium was composed of peptone water (consisted of peptic digestible animal tissue and sodium chloride) with addition of 0.2% potassium nitrate, distributed in test tubes, each with an inverted Durham tube and sterilized by autoclaving for 15 minutes at 121°C.

3.1.3.3 MRS broth

The medium had the same components of MRS agar mentioned above (3.1.3.1.5) without agar.

3.2 Methods

3.2.1 The preparation of Okah in laboratory

Okah was prepared by the most common method followed in Diling and Jebel Fenda. Okah is prepared in the laboratory using fresh heifer urine collected in plastic container and kept at room temperature in the laboratory for fermentation. The fermentation was continued for 8 days. Sampling was done every day for microbial analysis and three times during the fermentation for chemical analysis. Samples were taken at 0 time (raw material), after 5 days of fermentation (change in colour), and at the end of fermentation.
3.2.2 Microbiological methods

3.2.2.1 Preparation of serial dilutions

Ten ml of each sample were added to a conical flask containing 90 ml sterile peptone water. One ml of this mother solution (dilution $10^{-1}$) was pipetted aseptically into 9ml sterile peptone water to give $10^{-2}$ dilution and other serial dilutions were prepared in the same manner as described in Harrigan (1998).

3.2.2.2 Total viable count of aerobic microorganisms (TVC)

Total viable count was carried out using pour plate methods with plate count agar. From proper dilutions, one ml was transferred into the plate then the medium poured and mixed in. The plates were incubated at 37ºC for 2 days. The proper plates with 30-300 colonies were counted. Counting was carried out using a colony counter and expressed as cfu/ml for liquid samples (and cfu/ml during fermentation). Representative colonies of variety types were isolated from plate count agar, purified by repeated streaking on N.A. and stored as slope agar for further tests (Harrigan, 1998).

3.2.2.3 Counting of anaerobic microorganisms

Suitable dilutions of each of samples above were plated on plate count agar medium; the plates were incubated anaerobically at 37ºC for 48 hrs. Anaerobic conditions were created by incubation in jars equipped with $\text{H}_2+\text{CO}_2$ generating gas pack system as described by the manufacturer (Kiss, 1984).

3.2.2.4 Mould and yeast enumeration

From suitable dilutions of each sample 0.1ml was aseptically transferred onto solidified malt extract agar, the sample was spread all
over the plate using sterile bent glass rod and then the plates were incubated at 28°C for 48 hrs; the grown colonies were counted (Harrigan, 1998).

3.2.2.5 Staphylococcus enumeration

From each suitable dilution 1ml was transferred aseptically into sterile Petri dishes, then 15ml of Staphylococcus 110 medium were added and the inoculum was mixed with medium and allowed to solidify, the plates were then incubated at 37°C for 48 hrs and count was expressed as cfu/ml.

3.2.2.6 Enumeration of lactic acid bacteria

From suitable dilutions of each sample, 0.1 ml was transferred aseptically onto solidified MRS agar. The inoculum was spread all over the plate using sterile bent glass rod. The plates were incubated for 48 hrs at 37°C by using anaerobic jars and gas generating kits (Harrigan, 1998).

3.2.2.6.1 Identification tests of lactic acid bacteria

Representative single colonies showing morphological characteristic of LAB were picked from the plates used for viable count. These colonies were transferred to and purified on MRS agar. The pure colonies were tested for Gram stain and catalase reaction. The pure cultures of LAB were streaked on MRS agar slopes and stored at 5°C for identification.

3.2.2.6.1.1 Growth at 45°C

The isolates were tested for growth at 45°C according to Holt et al. (1994). Test tubes containing 9ml of MRS broth were used, each was inoculated with one ml of 24 hour old broth culture of each LAB
isolate. The inoculated tubes were incubated at 45ºC for 48 hour then
the turbidity of the broth indicated the growth of the bacteria.

3.2.2.6.1.2 Growth in 6.5% and 18% NaCl

Growth in the presence of 6. 5 and 18% NaCl was determined
according to Holt et al. (1994). MRS broth containing 6.5 or 18%
NaCl was inoculated with one ml 24 hour old broth culture of each
LAB isolate and incubated at 37ºC for 48hrs, then examined for
growth which is indicated by turbidity.

3.2.2.6.1.3 Growth at pH 4.4 and pH 9.6

The pH of MRS broth medium was adjusted to 4.4 and 9.6 with
the addition of drops of HCl and NaOH, respectively. The pH was
read by pH meter (Hanna PH 210 Microprocessor pH meter). Growth
turbidity was checked after 48 hrs incubation at 37ºC.

3.2.2.6.1.4 Production of acid and gas from glucose

The medium used was peptone water with the addition of 1%
glucose. An indicator, Andrade's, was used to detect acid production.
The medium was sterilized in autoclave at 110ºC for 10 minutes ,then
inoculated with 0.5 ml culture and incubated at 37ºC for 7 days .The
change in colour of indicator to pink means positive test (Harrigan,
1998).

3.2.2 Biochemical tests

3.2.2.1 Gram stain

A single colony of culture of each isolate was picked with
sterile loop, emulsified in a drop of distilled water on a clean slide and
spread out to make a thin film. The slide was air dried and the smear
was fixed by passing through a flame three times. The smear was then
stained with crystal violet solution for one minute and rinsed rapidly with tap water, Gram’s iodine solution was added and left for one minute, then poured off, the slide left to dry, washed with 95% ethanol, rinsed with tap water and stained with safranine for 30 seconds, then washed well and air dried. The smear was then examined microscopically by the oil immersion lens (Harrigan and McCance, 1978).

3.2.2.2 The staining of bacterial spores

A smear of each bacteria isolate was made as described above in Gram stain. The smear was flooded with malachite green solution and was placed over a water bath for 5 minutes. The slide was kept flooded with malachite green by adding the solution to the slide, then rinsed under tap water and stained with safranine for one minute. The slide was washed well and air-dried. The smear was examined microscopically by the oil immersion lens. The spores would be green and the cells red.

3.2.3.3 Motility test

A tube of motility medium, was inoculated with a 24 hrs culture. This was done aseptically using a straight wire, to half depth of the tube. During growth, motile bacteria will migrate from the line of inoculation to form turbidity in the surrounding medium, non-motile bacteria will grow only along the line of inoculation.

3.2.3.4 Catalase test

One ml of 3% solution of hydrogen peroxide (H₂O₂) was deposited on a clean slide and culture was added. The release of bubbles (of oxygen) indicated the presence of the enzyme catalase.
3.2.3.5 Oxidase test

With a loop, some bacterial growth was smeared on filter paper impregnated with tetra-methyl 1-p-phenylenediamine hydrochloride solution. A positive reaction would be indicated by purple colour after 10-15 seconds (Harrigan, 1998).

3.2.3.6 Oxidation /Fermentation (O/F) test

Fresh cultures (18-24 hours) were tested for O/F test by stab inoculation into pairs of freshly steamed Hugh and Leifson’s medium contained in test tubes. One of the tubes was sealed with sterile paraffin oil and the other unsealed. Incubation was carried out at 37ºC for 2-7 days. Acid production was shown by change in the colour of the medium from blue to yellow. but fermentative organisms produced acid in both tubes while oxidative organisms produced acid in the open tube only.

3.2.3.7 Voges-Proskauer test (V.P test)

This test was used for identification of Bacillus species. The medium used was glucose phosphate broth, distributed in 5ml amounts in test tubes and after inoculation with culture, the tubes were incubated at 37ºC for 3-7 days, then 0.5ml of 5% α-naphthol solution and 0.5 ml of 16% KOH solution were added and the tube shaken well. The development of a pink colour in the medium, usually within 30 minutes, indicated a positive reaction (Harrigan, 1998).

3.2.2.8 Starch hydrolysis

A poured, dried plate of starch hydrolysis medium was inoculated by spot inoculating on the surface and the plate incubated at 37ºC for 2-4 days. Gram’s iodine solution was added; unhydrolyzed
starch forms a blue colour with the iodine and areas of hydrolysis appear as clear zones.

3.3.3.9 Reduction of nitrate

Nitrate broth medium culture was incubated at 37ºC together with a sterile control tube for 2-7 days. After incubation one ml of each of two reagents was added to the culture. Reagent 1 consisted of sulfanilic acid and acetic acid 5N. Reagent 2 consisted of α-naphthol and ethanol. Red colour appearing within few minutes means positive test (Harrigan, 1998).

3.2.3.10 Haemolysis of blood agar

The culture was streaked on the surface of blood agar medium (see 3.1.3.1.7) and incubated at 37ºC for 24 hrs. Clear zones around the colonies indicated haemolytic activity (Harrigan, 1998).

3.3 Physiochemical methods

3.3.1 pH determination

The pH of Okah samples and fresh urine were read directly using pH meter (Hanna pH 210 Microprocessor pH meter).

3.3.2 Moisture content

The moisture content of samples was determined using the method cited in AOAC (1990). Two Gram’s of each of the Okah and urine samples were dried in crucibles in air-dry oven at 100-103ºC over-night, then dried in desiccators. The moisture content was calculated as follows:

\[
M\% = \frac{(\text{Crucible wt + sample wt}) - (\text{crucible wt+ sample after drying})}{\text{Sample wt}} \times 100
\]
3.3.3 Crude fat (C.F)

The fat content was determined by Gerber method according to AOAC (1990) as follow: ten ml sulfuric acid (density 1.86mg/ml at 20°C) were poured into a clean Gerber tubes, then thoroughly mixed with sample till no white particles were seen, then centrifuged at 1100 revolution per minute (rpm) and transferred to water bath at 65°C for 3 minutes, the columns of the fat was then recorded immediately.

3.3.4 Nitrogen and crude protein (C.P)

The protein content was determined by Kjeldhal method (AOAC, 1990). Ten ml of the sample were placed into Kjeldhal flask. Then two kjeldhal tablets each containing 1g Na₂SO₄ and 0.1g Hg. Twenty five milliliters of concentrated sulfuric acid (density of 1.86mg/ml at 20°C) were added to flask. The mixture was digested on a heater for 2-5 hours until the colour was cleared. The flask was removed and left to cool. Then constituents were diluted to 75ml volume using distillation. The ammonia in the distillate was determined by titration against 0.1N HCl until the end point was obtained (red colour).

Protein content was calculated as follows:

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

Where:

- \(T\) = Titration figure
- 0.1 = Normality of HCl
- 14 = Equivalent weigh of nitrogen
- 20 = Dilution factor

\[
\text{crude Protein} = \text{N}\% \times 6.26
\]

Where

6.26 = Protein conversion factor
3.2.5 The ash content

The ash content was determined according to AOAC (1990). Two gram’s of Okah and urine were weighed into a suitable clean dry crucible and evaporated to dryness on steam bath and the crucible was placed in a muffle furnace at 550°C for 4 hours, cooled in a desiccator and weighed. The ash content was calculated as follows:

$$\text{Ash\%} = \frac{(W_2 - W_1) \times 100}{S}$$

Where:

- $W_1$ = Weight of empty crucible
- $W_2$ = Weight of crucible after ashing
- $S$ = Weight of sample

3.3.5.1 Analysis of inorganic matter

Minerals of raw and processed samples were extracted according to Pearson’s method (1981). Each sample was burnt in a muffle furnace at 550°C. Each sample was placed in a sand bath for 10 minutes after addition of 5ml of 5N HCl. Then the solution was carefully filtered in 100ml volumetric flask and finally distilled water was added to make up to 50 ml. The extracts were stored in bottles for further analysis.

3.3.5.1.1 Determination of sodium and potassium

Sodium and potassium contents of each extracted sample were determined according to AOAC, (1990) using flame-photometer (Corning 400). One milliliter of extract was taken and diluted in 50ml distilled water. The standard solution of the NaCl and KCl were prepared by dissolving 3.33, 2.54 g of NaCl and KCl respectively in one liter distilled water. Ten ml of this solution were taken and diluted to 1000ml by addition of distilled water to give 10 ppm concentration.
The flame photometer was adjusted to zero using distilled water as a blank and to 100 using standard solution.

Calculation:

$$\text{Na or K} \% = \frac{\text{F.R.} \times \text{D.F.}}{10 \times \text{S} \times 10 \times \text{eq.wt}}$$

Where:

- F.R = Flame photometer reading
- D.F = Dilution factor
- S = Sample weight
- Eq.wt = Equivalent weight of Na = 23, K = 39

### 3.3.5.1.2 Determination of phosphorus

Analysis of phosphorous was carried out according to the method of Champman and Pratt (1961). Two milliters of the extract were pipetted into 50 ml volumetric flask. Ten milliters of ammonium molybdate (Ammonium vanadate reagent (22.5g of (NH₄)₆MO₇O₂4H₂O) in 400ml distilled water +1.25g ammonium vanadate in 300ml boiling water +250 ml concentrated HNO₃, then diluted to one liter) were added. The contents of the flask were mixed and diluted to volume (50 ml). The density of the colour was read after 30 minutes at 470 nm using spectrophotometer (JENWAY model 6300). A standard curve of different KH₂PO₄ concentrations was plotted to calculate the ion phosphorous concentration.

Calculation:

$$\frac{\text{Reading curve} \times \text{ash dilution} \times 50\text{ml}}{10 \times \text{oven dry weight of sample}}$$

### 3.3.5.1.3 Determination of calcium, magnesium, iron and zinc

Minerals Ca, Mg, Fe, and Zn were determined using atomic absorption spectrophotometer (AA-6800, Shimadzu, Japan). The wave lengths 248.3, 213.9, 422.7 and 285.2nm, were used
respectively. Standard curve was prepared for each one mineral with the values presented on the x axis and absorbance presented on the y axis. A blank was prepared from HCl plus distilled water. Then the mineral content of each sample was read from standard curve. Samples were not determined at the same time, therefore a fresh standard curve was used for each determination.

3.3.5.1.4 Determination of total protein in urine

Urinary protein was determined by the method of Biuret reaction as described by Weichselbaum (1964). The protein was concentrated by precipitation using trichloroacetic acid. It was redissolved in an alkali and measured spectrophotometrically at 420 nm.

3.3.5.1.5 Determination of non-protein nitrogen (Urea, creatinine)

The urea was determined by the manual method described by Evans, (1968) and March et al. (1965). The urine sample was diluted 1 : 10 with distilled water. The possible proteins were precipitated with trichloroacetic acid and centrifuged, the sample absorbance was read (standard 10mmol/L at 520 nm).

The creatinine was determined by the method of alkaline picrate of Bosnes and Toussky, (1945). One ml of urine sample was completed to 100 ml with distilled water, 1 ml picric was added to 3 ml diluted urine, followed by 1 ml of NaOH. Three ml creatinine standard (0.3 micromol and 3 ml distilled water) were used as blank were treated the same way. The absorbance was measured after 15 minutes at 500 nm.
3.3.5.1.6 Determination of Uric acid

Uric acid was estimated according to Brown (1954). Five ml of diluted urine (1:100), 1ml of 10% sodium tungestate and 1ml of 2N H$_2$SO$_4$ were mixed and centrifuged. Five ml of the supernatant were put in one tube and another tube contained the blank. To each tube, 1ml of 10% sodium carbonate and 1ml of phosphatungestic acid were added and left to stand at room temperature for 30 minutes, then were read at 680 nm.
 Calibration curve of Mg
Straight line curve formula
Abs = 1.00564 conc + 0

Abs = absorbance
Fig 2

Calibration curve of Ca
Abs=0.0377402 Conc+0

Abs = absorbance
Fig 3

Calibration curve of Fe
Abs=0.0652198 Conc+0

Abs = absorbance
Calibration curve of Zn

Abs = 0.559022 Conc + 0

Abs = absorbance
4.1 Microbiological aspects of Okah

4.1.1 Microbial counts of collected Okah samples

Microbial count was determined for eight Okah samples collected from ElDiling and Elfenda homes (Table 1). All samples (A-H) are one year old except C and G were four months old. The collected samples were made of pure fermented urine except sample C in addition to urine contained corn. Many of Elfenda people prefer the addition of corn to urine. The highest counts of aerobic microflora were found in samples C and G, which were $5.1 \times 10^5$ and $6.8 \times 10^5$ cfu/ml respectively. These two samples are 4 months old and considered recent, compared to the rest of the samples. The lowest count of aerobic microflora was recorded in sample B which was $3.1 \times 10^2$ cfu/ml. Anaerobic counts of viable microflora were higher in sample C ($3.5 \times 10^4$) and F ($3.2 \times 10^4$ cfu/ml). No anaerobic growth was recorded in sample B. Generally the TVC of aerobic microflora was higher than the TVC of anaerobic microflora.

Plate 1 shows the variation in the colour of the samples. Samples colour varied from yellow, brown to black, depending on the age of the sample. This may be due to period of fermentation, microbial enzymes or urine minerals or oxidation due to exposure to air.

Elbashir (2004) reported that colour intensity of urine, ranged from colourless to slight yellow and watery for human and younger animals and amber yellow to ferrous in case of adult camel. On long standing the colour may change to deep brown or red.
Table 1: Total viable count (cfu/ml) of aerobic and anaerobic microflora of Okah samples

<table>
<thead>
<tr>
<th>Test Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
</tr>
<tr>
<td>B</td>
</tr>
<tr>
<td>C</td>
</tr>
<tr>
<td>D</td>
</tr>
<tr>
<td>E</td>
</tr>
<tr>
<td>F</td>
</tr>
<tr>
<td>G</td>
</tr>
<tr>
<td>H</td>
</tr>
</tbody>
</table>
Plate 1: The variation in colour of Okah samples
Although yeast didn't grow up in the specific media for yeast, some colonies on plate count agar proved to be yeast colonies as in case of sample A and C.

4.1.2 The total count and pH from heifers and milking cow urine

Two urine samples A and B collected from a milking cow and heifer respectively were examined. The milking cow urine was clear yellow with pH 7.64 and the heifer urine was slightly yellow with pH 7.63 (Plate 2). The milking cow urine was found to have high microbial load (4.3×10^3 cfu/ml) than the heifer urine (3.1×10^2 cfu/ml) (Table 2).

4.1.3 Microbial counts of four months and one year old samples in medium composed of different urine concentrations

The growth of microorganisms of the different Okah samples in plate count Agar medium was very weak. Therefore, to enhance the growth of the microorganisms the medium ingredients were dissolved in water and urine at different ratios. The media were prepared using distilled water and urine in the ratio 200:0, 180:20, 160:40, 140:60, 120:80, 100:100, 80:120, 60:140, 40:160, 20:180 and 0:200. Figure 1 shows the growth of microorganisms in these media prepared using these ratios.

The highest count (7.4x10^6) was obtained in the medium prepared in the ratio 1:1 and the lowest count (4.5x10^5) was obtained with the medium prepared in the ratio 180:20. That means the suitable ratio for the growth of microorganism is that containing equal volumes of water and urine. It is clear that the presence of urine in the medium enhanced the growth of microorganisms. It could be stated that urine supplemented the medium with some nutrients which may be lacked in the artificial medium.
Plat 2: Cow (A) and heifer (B) fresh urine samples
Table 2: The total viable count and pH of heifer and milking cow fresh urine

<table>
<thead>
<tr>
<th>Samples</th>
<th>Test</th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total viable count</td>
<td></td>
<td>$4.3 \times 10^3$</td>
<td>$3.1 \times 10^2$</td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td>7.64</td>
<td>7.63</td>
</tr>
</tbody>
</table>
Fig (1): Microbial count from sample four month and year microflora in medium composed of different urine concentration
A=one year old sample
B= four month old sample
4.1.4 Microbial count during the laboratory fermentation of Okah

Fresh urine was obtained from cows in Shambat farm. The laboratory fermentation of Okah took eight days. During this period sampling was done daily for enumeration of microorganisms. The total viable count of microorganisms was done every day (24 hours). The aerobic viable counts of various microorganisms during Okah fermentation is shown in Table (3). The initial aerobic counts at day 0 were $1.7 \times 10^5$, $3.1 \times 10^3$ and $2.93 \times 10^5$ for TVC, *staphylococcus*, *Lactic acid bacteria* respectively and no growth of yeast and mould was observed (Table 3; Fig 2).

Total viable counts (TVC) of the fresh urine sample was $10^5$. This viable count increased to $10^6$ after two days of fermentation. Then counts decreased to $6.7 \times 10^6$, in day 4, $4.6 \times 10^5$ in day 5, $4.2 \times 10^3$ in day 6 and no growth was obtained in the remaining days of fermentation.

*Staphylococcus* bacteria count of fresh sample was $3.1 \times 10^3$. The counts reached $3.9 \times 10^3$ in the first day of fermentation, and started to decrease after the third day. This decrease in the *Staphylococcus* count is attributed to rise in the pH.

Sinskey and Nickerson (1974) reported that range of pH that permits the growth of *Staphylococcus* is 4.8-7.6.

Lactic acid bacteria count started with $2.93 \times 10^5$ at zero time, $2.87 \times 10^5$ day 1, then decreased to $1.1 \times 10^4$ in day 2 to increase again in day 3 to $1.44 \times 10^7$ and no growth in the other days of fermentation (less than 30 colonies).
<table>
<thead>
<tr>
<th>Day</th>
<th>Fermentation</th>
<th>TCV</th>
<th>Staphylococcus</th>
<th>Lactic acid bacteria</th>
<th>Yeast and mould</th>
<th>PH</th>
</tr>
</thead>
<tbody>
<tr>
<td>60’8</td>
<td>Nil</td>
<td>1.7x10^5</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>7.49</td>
</tr>
<tr>
<td>60’8</td>
<td>Nil</td>
<td>2.95x10^6</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>7.46</td>
</tr>
<tr>
<td>8’03</td>
<td>Nil</td>
<td>4.9x10^4</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>7.66</td>
</tr>
<tr>
<td>8’08</td>
<td>Nil</td>
<td>1.01x10^7</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>7.83</td>
</tr>
<tr>
<td>7’46</td>
<td>Nil</td>
<td>6.7x10^6</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>8.17</td>
</tr>
<tr>
<td>7’49</td>
<td>Nil</td>
<td>4.6x10^5</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>8.08</td>
</tr>
<tr>
<td>8</td>
<td>Nil</td>
<td>4.2x10^3</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>8.05</td>
</tr>
<tr>
<td>7</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>8.09</td>
</tr>
<tr>
<td>6</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>8.09</td>
</tr>
</tbody>
</table>

Table 3: Changes in viable counts (cfu/ml) of microbial groups during laboratory fermentation of Okah.
Fig 2: Changes in count of microbial populations during the laboratory fermentation of Okah

a= TVC  
b= Staphylococcus  
c= Lactic acid bacteria  
e= Yeast and moulds
There is no yeast and moulds growth during the whole period of fermentation. This may be due to the alkaline nature of cow urine. During the laboratory fermentation of Okah the pH of urine did not drop to acidic levels.

The pH during fermentation was 7.49 at zero time, 7.46 day 1, 7.66 day 2, 7.83 day 3, 8.17 day 4, 8.08 day 5, 8.09 day 6, 8.09 day 7 and 8.

The pH of the fermenting urine increased (Fig. 3). It increased during the first four days of fermentation then showed a slight decrease in the remaining days of fermentation.

Elbashir et al. (2004) reported that the camel and cattle urines were alkaline with an average of 9.5, 8.6, respectively while the goat and human urine are acidic with average of pH 4.85, 6.5 respectively. This variation in pH was attributed to feed intake (high energy diets), such diets can cause metabolic acidosis, mineral distribution and environmental conditions.

4.1.5 Biochemical identification of bacterial isolates

4.1.5.1 Tentative identification of rod-shaped bacteria isolated from aerobic and anaerobic plates of Okah samples

Identification of the isolates was based on biochemical tests. Ten rod-shaped bacteria were isolated aerobically from Okah samples in Table (4). The tests revealed that seven isolates were *Bacillus* and three were *Corynebacterium*. The Bacillus isolates, two are *Bacillus coagulans*, two *Bacillus brevis* and one isolate *Bacillus pumilis*, *Bacillus licheniformis* and *Bacillus macerans*. These results indicated that the urine fermentation is dominated by the species of the genus Bacillus.
Fig 3: The pH during fermentation of Okah (Data from Table 3)
Table 4
Eighteen isolates of rod shaped bacteria were isolated anaerobically (Table 5). These isolates were definitely *Bacillus*, because the results of identification showed Gram stain, spore, catalase, oxidase, motility and (O/F) test were positive and not *Clostridium* because they also grew under aerobic conditions.

Fourteen isolates were *Bacillus* and four isolates were *Corynebacterium*. Nine isolates were found to be *Bacillus coagulans*, three isolates were *Bacillus licheniformis* and two isolates were *Bacillus macerans*.

Elkhalifa (2003) reported in the study of the camels urine, the present bacteria were *Bacillus* spp and *Corynebacterium*.

Tannock (1995) reported that some of the *corynebacteria* are obligate aerobes, others are facultative anaerobes. They produce enzymes that degraded lipids, urea and proteins.

Corry *et al.* (1999) reported that most aerobic endospore forming bacteria (AEFB) will grow on nutrient agar. A few species require glucose or other carbohydrates in nutrient agar or have more specific nutritional requirements such as uric acid.

Thermotolerant species of *Bacillus* able to grow at 55°C may be isolated from wide range of environments like soil, sewage, compost, water or dust. With exception of *Bacillus coagulans* which prefers a more pH range for growth and its lower acid tolerance, *Bacillus licheniformis* is able to grow anaerobically and can tolerate up to 15% NaCl, as this organism can reduce both nitrate and nitrite with the production of gas.
Table 5
4.1.5.2 Tentative identification of coccus bacteria isolated aerobically and anaerobically from Okah samples

Ten isolates of coccus bacteria were isolated aerobically from Okah samples. The results showed that nine isolates were *Staphylococcus* spp. and one isolate was *Micrococcus*. The *Staphylococcus* isolated aerobically, two isolates were *Staphylococcus aureus* and seven were *Staphylococcus albus* (Table 6).

Anaerobically eleven isolates of *Staphylococcus* spp were isolated. Seven of the *Staphylococcus* isolates belong to *Staphylococcus aureus* and four belong to *Staphylococcus albus* (Table 7).

Both micrococci and staphylococci demonstrate positive catalase activity. The two genera *Micrococcus* and *Staphylococcus* can easily be separated based on the oxidation-fermentation (O/F) test. *Micrococcus* is obligate aerobe and produces acid from glucose only aerobically, where *Staphylococcus* is facultative aerobe and produces acid from glucose both aerobically and anaerobically.

The strain which usually produces white pigmented colonies is classified as *Staphylococcus albus*, while that produces golden yellow is *Staphylococcus aureus* (Madigan et al, 1997; Gillies and Dodds, 1973).

Madigan et al (1997) reported that Gram-positive cocci are relatively resistant to reduced water potential and tolerate drying and high salt.

Elkhalifa (2003) reported the existence of the Gram-positive bacteria in camels urine were identified as *Staphylococcus* ssp and *Micrococcus*. 
Table 6
Table 7
4.1.5.3 Tentative identification of microflora of fresh urine in samples (A and B)

The microorganisms from sample A (fresh milky cow urine) and sample B fresh heifer urine) were shown in Table 8. A total of 20 isolates was obtained, thirteen isolates from sample A and seven isolates from sample B.

Eight isolates out of the 13 isolate of sample A were cocci and five were rod. The cocci four were Staphylococcus and two Micrococcus and two Streptococcus, the rod were three isolates Bacillus and two isolates Corynebacterium.

The isolates obtained from the heifer urine two cocci were Staphylococcus and Micrococi, and four rod isolates were two Bacillus and two Corynebacteria.

4.1.5.4 Tentative identification of bacteria of 4 months and one old year samples in different concentrations of fresh cow urine:

The aerobically isolated bacteria from both samples (four month old and one year old) were twenty six isolates in Table (9). Out of these isolates fifteen were obtained from the four months old sample and the remaining from the one year old sample. Those from the four months old sample contained Staphylococcus, Micrococcus, and Corynebacterium, while those of the one year old sample contained Staphylococcus, Micrococcus, and Bacillus.

Actinomycetes growth was recorded under aerobic conditions. The identification tests showed that these isolates were filamentous, Gram positive, non-sporing, non-motile, catalase and oxidase positive and O/F positive.
Table 8
Table 9
4.1.6 Tentative identification of microorganisms isolated on PCA during the laboratory fermentation

The isolates obtained during fermentation of urine (Okah) in the laboratory are shown in Table 10. A total of thirty isolates was obtained, twenty-five isolates were isolated aerobically and five anaerobically. Out of the thirty isolates twenty-two were \textit{Staphylococcus}, eight isolates were \textit{Micrococcus} and four isolates were \textit{Bacillus}.

Hazare (2005) reported that the microorganisms found in normal urine are \textit{saphylococci} and \textit{bacili}.

4.1.7 Tentative species identification of \textit{Staphylococcus} bacteria on staph. 110 medium during the laboratory fermentation of urine (Okah)

Isolation of \textit{Staphylococcus} species was done on \textit{Staphylococcus} No 110 medium. Tests for the presence of \textit{Staphylococcus} were carried out during the laboratory fermentation. The results were that ten isolates were found to be \textit{Staphylococcus}. These isolates belonged to \textit{Staphylococcus aureus}, \textit{Staphylococcus albus} and \textit{Staphylococcus epidermidis} in Table (11).plate 3.

The strains which usually produce white colonies classified as \textit{Staphylococcus albus}, while that produce golden yellow \textit{Staphylococcus aureus}, \textit{Staphylococcus albus} and \textit{Staphylococcus aureus} are coagulase -positive (Gillies and Dodds, 1973), while \textit{Staphylococcus epidermidis} is coagulase negative (Madigan et al, 1997).
Table 10 contd.
Table 11
4.1.8 Tentative genus identification of lactic acid bacteria isolated during laboratory fermentation of urine (Okah)

Lactic acid bacteria isolated during the laboratory fermentation of Okah were identified and the results are given in Table (12). Eight isolates were identified as Lactobacillus and seven isolates as Enterococcus.

Lactic acid bacteria (LAB) is a group of bacteria that give many fermented foods stability.

Lactic acid bacteria are generally fastidious on artificial media, but they grow readily in most food substrates and lower pH. Their growth and fermentation is affected by the temperature and salt concentration (Steinkraus, 1992).

Enterococcus is homo-fermentative while some lactobacilli are homo-fermentative and others are hetero-fermentative and produce carbon dioxide are a group of bacteria that give many fermented foods stability (LAB).

4.2 Physiochemical properties

4.2.1 The chemical composition of fresh cow urine and heifer

The chemical composition of fresh urine of cow and heifer is shown in Table 13. Sample A (cow urine) has moisture content 94.29%, protein 4.5%, fat 0.01% and ash 1.2%

That of the heifer (sample B) has moisture content of 94.09%, protein 4.5%, fat 0.01% and ash 1.4%.

The results of milking cow and heifer urine revealed no differences in composition of cow and heifer urine. The microbial analysis of both urine showed that the cow urine has higher microbial load than that the heifer. This may be one of the reasons that
Table 12
Plat 3: The colonies of Staphylococcus bacteria
Table 13: The chemical composition of fresh urine of milking cow and heifer

<table>
<thead>
<tr>
<th>Test</th>
<th>Sample</th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture%</td>
<td></td>
<td>94.29</td>
<td>94.09</td>
</tr>
<tr>
<td>Crude protein</td>
<td></td>
<td>4.5</td>
<td>4.5</td>
</tr>
<tr>
<td>Ether extract (fat%)</td>
<td></td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Ash%</td>
<td></td>
<td>1.2</td>
<td>1.4</td>
</tr>
</tbody>
</table>
the Nuba people prefer heifer urine for making Okah.

4.2.2 Composition of okah samples collected from Diling and Fenda Tribe

Okah a fermented cow urine used as a food in Diling area of Nuba Mountains. Little or no literature is available with regard to the constituents of Okah.

The chemical analysis of Okah were done for eight samples as shown in Table (14). The results of these analyses showed that Okah contains a high moisture that range between 87.36 to 96% with an average of 92.95%.

Protein content ranged between 1.09 to 6.04% with an average of 2.98%. Fat content ranged between 0.1 to 1% with an average 0.25%.

Ash content ranged between 2.6% to 6.4% with an average of 3.82% (Figs. 4, 5, 6, 7) respectively.

Potassium, sodium, nitrogen and phosphorus were determined. The results showed that 1.00 to 2.30% with an average 1.66, 0.1 to 0.2% with an average 0.11, 0.7 to 1.93% with an average 1.21, 0.05 to 0.26% with an average 0.16% respectively.

Calcium, magnesium, zinc and ferrous results were 1.03 to 9.9% with an average 4.9, 1.02 to 1.9% with an average 1.45, 1.5% to 3.3% with an average 2.05% and 1.1 to 6.2% with an average 3.30% respectively.

Sample A showed a very high content of protein, fat, ash and potassium that may be due to addition of starter. Because, the fermentation of starter usually took along time. These may be referred to biological enzymes reaction of microbes.
Table 14
Fig 4: Moisture content of the different Okah samples (Data from Table 13)

Fig 5: Protein content of the different Okah samples (Data from Table 13)
Fig 6: Fat content of the different Okah samples (Data from Table 13)

Fig 7: Ash content of the different Okah samples (Data from Table 13)
Some of Okah sample as E and G were similar in most of the constituent.

The composition of urine depends on many factors. These include Sweating, environment, animal feeding and season.

The type of drinking water used for animal feeding, saline water increased the urinary and electrolyte excretion compared to animal using fresh water. Eltayeb (2006) reported that the increase in urine output of Na and Cl and the decrease in urinary K associated with drinking water of 1.7% salt.

The excretion of Ca, Mg, K, P, Na and Cl was increased by saline water (0.8 - 1.3% NaCl) ingestion, while the faecal excretion of Ca, Mg and P was not affected by the inclusion of sodium chloride in the drinking water.

Total nitrogen in the cow urine ranged from 6.8 to 21.6gm/liter (Birstow et al., 1992), of which an average of 69% was present as urea.

4.2.3 Proximate composition of Okah produced by laboratory fermentation

The chemical composition of Okah produced by the laboratory fermentation is shown in Table 15.

The chemical analysis of fresh urine recorded that, moisture content was 93.1%, protein was 4.5%, fat 0.01% and ash content was 1.4%

At day five during the laboratory fermentation the colour was changed from yellow to brown. At this stage the chemical analysis was carried out. The moisture content was 93.6%, protein was 6.3%, fat 0.01%, and ash 0.09%.

At the last day of fermentation, moisture content was 90.1%, protein was 10.7%, fat 0.01% and ash content 0.67%.
Table 15: Proximate composition of Okah produced by laboratory fermentation

<table>
<thead>
<tr>
<th>Test</th>
<th>Fresh urine (day 0)</th>
<th>Day (5)</th>
<th>Last day (8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture%</td>
<td>93.1</td>
<td>93.6</td>
<td>90.1</td>
</tr>
<tr>
<td>Crude protein</td>
<td>4.5</td>
<td>6.3</td>
<td>10.7</td>
</tr>
<tr>
<td>Ether extract fat%</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Ash%</td>
<td>1.4</td>
<td>ND</td>
<td>0.67</td>
</tr>
</tbody>
</table>
This study indicated considerable increase in the protein content of the Okah compared to the fresh urine.

4.2.4 The chemical composition of cow, heifer, ox and fermented urine

Table (16) shows the chemical composition of cow, heifer, Ox and fermented urine. The total protein of the different animals were close to each other; it ranged from 7.7 to 8.1. Similar results were obtained for the different component such as urea, uric acid, Na, K, Ca, P and albumin. With regard to sodium content the fermentation of urine showed high Na content compared to the other type of urine.

Urea is the major end product of nitrogen metabolism

The amount of urea in the different type of urine were 18, 17, 19 and 16 (mg) for cow, heifer, ox and fermented urine respectively.

These results were higher than those reported by Elbashier (2004) who reported 14.2 urea, 18.2 uric acid and 127.17 creatinine for cattle urine.
Table 16
CHAPTER FIVE
CONCLUSION AND RECOMMENDATIONS

5.1 Conclusions

Okah is fermented fresh cow urine. It contains 2.98% protein, 0.25% fat and 3.82% ash.

Microbiological analysis of Okah showed that it contains Bacillus coagulans, Bacillus licheniformis, Bacillus brevis, Bacillus macerance, Staphylococcus spp, Micrococcus spp, Corynebacterium spp and some yeast and actionmycetes.

The main microorganisms responsible for fermentation of Okah were Bacillus, Corynebacterium, Staphylococcus, Micrococcus, Lactobacilli and Enterococcus.

Although there is no difference in the chemical composition of cow and heifer urine, the cow urine has higher microbial load than heifer.

5.2 Recommendation

1. Future research is recommended for comparison between the fermented cow and heifer urine.
2. More investigation of microorganisms of okah using advanced molecular techniques is recommended.
3. This product is rich in minerals and contain high protein.
4. The use of more precise chemical analysis technique is recommended for the analysis of okah.
REFERENCES


