Nutritional Value and Microbiological Quality of (Agashi) Product

القيمة الغذائية والجودة الميكروبيولوجية لمنتج الأقاشي

By

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بسم الله الرحمن الرحيم

تَدْعِيم

جَهَّازَةٌ تَدْفَعَ جِيْشًا

صدق الله العظيم

( سورة الواقعة الآية 21)

DEDICATION
To My:
Family,
Teachers and Friends.

ACKNOWLEDGEMENTS

With all due humbleness and gratitude I render ultimate thanks and special praise to GOD (Almighty) who gave me health, power and patience to accomplish and conduct this work.

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ABSTRACT

This study aimed to determine the chemical composition, nutritional value and microbiological quality of Agashi sliced meat product which is consumed largely as snack food in Sudan. The samples were taken from three different locations in Khartoum state (Omdurman, Khartoum and Khartoum North) from open and closed areas. The chemical composition of Agashi sliced meat was investigated. The results revealed that, Agashi sliced meat containing moisture (38.67%-35.74% - 35.14%and39.83% - 39.83% - 38.74%) in open and closed area in Omdurman, Khartoum and Khartoum North, respectively. Fat content (5.01% - 5.45% - 4.89% and5.76% - 6.09% - 5.96%) in open and closed area in Omdurman, Khartoum and Khartoum North, respectively. Proteins content (30.6% - 26.25% - 18.4% and 14.16% - 15.75% - 22.7%) in open and closed area in Omdurman, Khartoum and
Khartoum North, respectively. Ash content (3.07% - 2.75% -3.38% and 3.77% - 3.79% - 3.79% - 5.32%) in open and closed area in Omdurman, Khartoum and Khartoum North, respectively. Fibre content (11.39% - 4.39% - 3.81% and 10.41% - 5.16% - 4.90%) in open and closed area in Omdurman, Khartoum and Khartoum North, respectively. Carbohydrates content (40.05% - 43.29% - 52.03% and 48.83% - 40.39% - 51.85%) in open and closed area in Omdurman, Khartoum and Khartoum North, respectively.

The microbiological analyses revealed the presence of high levels of total viable count, coliforms, Staphylococcus aureus, E. coli, yeast and moulds in open area samples cooked Agashi product; moreover, the results revealed presence of salmonella in open area comprised to closed areas. This high contamination of cooked Agashi with food spoilage organisms and pathogens decrease the quality and it causes a public health hazard. Sensorial properties showed significant differences between open and closed area samples. Omdurman area samples had lowest acceptability according to panelists.

ملخص البحث

هذه الدراسة أجريت تحديد المكونات الكيميائية، القيمة الغذائية والجودة الميكروبيولوجية لشرايحة الأقاشي المستهلك، بالنسبة كبيرة في السودان. تم أخذ العينات من ثلاثة أماكن مختلفة بولاية الخرطوم (أمدرمان، الخرطوم، الخرطوم بحري) من أماكن مفتوحة وغلقية.

تم دراسة المكونات الكيميائية لشرايحة الأقاشي، وقد أظهرت النتائج أن شرايحة الأقاشي تحتوي على نسبة رطوبة (38.7% - 38.7% - 35.74% و 35.14% - 39.86% - 39.83% في الأماكن المفتوحة والمغلقة في أمدرمان، الخرطوم، الخرطوم بحري،على التوالي.

نسبة الدهن (5.01% - 5.45% - 4.89% و 5.76% - 6.09% - 5.96% في الأماكن المفتوحة والمغلقة في أمدرمان، الخرطوم، الخرطوم بحري،على التوالي.

نسبة البروتين (30.6% - 26.25% - 18.4% - 14.16% - 15.75% و 22.7% - 18.4% في الأماكن المفتوحة والمغلقة في أمدرمان، الخرطوم، الخرطوم بحري،على التوالي.

نسبة الزرادة (3.07% - 2.75% - 3.38% و 3.77% - 3.79% - 5.32% في الأماكن المفتوحة والمغلقة في أمدرمان، الخرطوم، الخرطوم بحري،على التوالي.

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نسبة الألياف (11.39% - 3.81% - 10.41% - 5.16% - 4.90%) في الأماكن المفتوحة والمغلقة في أمدرمان. الخطروم، الخطروم بحري، على التوالي.

نسبة الكربوهيدرات (52.03% - 43.29% - 40.05% - 48.83% - 40.39% - 51.85%) في الأماكن المفتوحة والمغلقة في أمدرمان، الخطروم، الخطروم بحري، على التوالي.

أيضا، أظهرت نتائج التحليل الميكروبي لهذه العينات وجود مستويات عالية من العد البكتيري الكلي. بكتريا الفولون، الخمار، والاعفان في الأماكن المفتوحة. وقد أظهرت هذه النتائج وجود السالمونيلا في الأماكن المفتوحة مقارنة مع الأماكن المغلقة.

هذا التلوث العالي للناقاشي المطبوع بالميكروبات المفسدة و الممرضة أدى إلى تقليل جودة المنتج وسلامة الغذاء وجعله غير ملائم للاشترابات الصحية.

الخواص الحساسة أظهرت وجود فروقات معنوية بين عينات الأماكن المفتوحة والمغلقة. عينات أمدرمان حصلت على نسبة قبول أقل من قبل المحكرين.
CHAPTER ONE

1. INTRODUCTION

Meat is defined as those animal tissues, which are suitable for use as food and it is often widened to include, as the musculature, organs such as liver and kidney, brains and other edible tissues (Lawrie, 1991). Meat and meat products are concentrated sources of high quality protein, and their essential amino acids content usually compensate for deficiencies in diets made mainly of cereals and other vegetable proteins. They supply easily absorbed iron and assist in the absorption of iron from other foods as well as zinc, and are rich sources of some of the B-vitamins. By providing such nutrients, meat consumption can alleviate common nutritional deficiencies (Bender, 1992). Rich nutrient matrix meat is the first-choice source of animal protein for many people all over the world (Heinz and Hautzinger, 2007). Consumption of meat is continuously increasing worldwide. The annual per capita consumption increased from 10 kg in the 1960s to 26 kg in 2000 and will reach 37 kg by the year 2030 (Heinz and Hautzinger, 2007). The activities of the meat sector may be divided into three stages: slaughtering, meat cutting and further processing. Each stage involves completely different technical operations which must not be viewed as separate and independent processes (FAO, 2007). Meat and meat products are highly perishable which spoil easily, soon become unfit for human consumption and possibly dangerous to health through microbial growth, chemical changes and breakdown by endogenous enzymes (Judge et al., 1990). Meat is highly perishable, because it is high in protein and moisture and semi-neutral in pH which makes it an ideal medium for bacterial growth (Warriss, 2000). Food safety is one of the most important issues in marketing any kind of food, especially meat (Barbut, 2005).

The most serious meat safety issues resulting in immediate consumer health problems and recalls from the market place of potentially contaminated products, are associated with organisms especially bacterial pathogens (Sofos, 2008). Meat
safety during processing, packaging, sorting, transporting, displaying, selling, cooking, serving and eventually consumption ideally should be constantly under tight scrutiny by government officials, food processors, food handlers, food providers, and the consumers themselves (Hui et al., 2001). The advantage of meat processing is the integration of certain animal tissues (muscle trimmings, bone scraps, skin parts or certain internal organs which are usually not sold in fresh meat marketing) into the food chain as valuable protein-rich ingredients (Heinz and Hautzinger, 2007). Sudan has huge animal wealth, estimated to be more than 104.9 million head, and classified as follows: 29.8 millions head cattle, 4.8 millions camel, 39.5 millions sheep and 30.8 million goats; the latest report indicates a population of about 140 million (Ministry of livestock, Fishers and Rangelands, 2013). Here in Sudan there are many types of foods handled differently. Some of them are sold in an open area while others are sold in closed area. One of these foods is Agashi, usually from beef with some additives. It was made of a number of finely ground spices including ginger, cinnamon, black pepper, salt, fennel and coriander. Crushed peanuts and bread crumbs were added to the mixed spices. Then beef meat (1/2 kg) was chopped to make small slices, and soaked in water for few minutes. The meat slices were placed in thin iron sticks, mixed with doga (mixed spices) and cooked l the colour of meat was changed to brown (Sulieman et al., 2012)

Main objective:
To study the nutritional value and safety of Agashi

Specific objectives:

1- To determine the proximate chemical composition of the product.
2- To find out the levels of different microorganisms associated with the product.
3- To determine the presence of the pathogenic microorganisms in the food.
CHAPTER TWO

2. LITERATURE REVIEW

2.1 Meat definition

Meat is defined as ‘the edible part of the skeletal muscle of an animal that was healthy at the time of slaughter (CFDAR, 1990).

FSANZ (2002) defined meat as the whole or part of any buffalo, cattle, deer, pig, poultry, rabbit or hare slaughtered other than in a wild state. This definition does not include eggs or fetuses. The term, meat refers only to meat flesh (skeletal muscle plus any attached muscle connective tissue or fat), but the FSANZ definition also includes offal's (i.e. meat other than meat flesh, including brain, heart, kidney, liver, pancreas, spleen thymus, tongue and tripe), and excludes bone marrow. The processed meat product are defined as those in which properties of fresh meat have been modified by use of one or more procedures, such as grinding or chopping, addition of seasoning, alteration of color or heat treatment. Generally, meat processing developed soon after people become hunter (Judge et al., 1990).

2.2 Chemical composition and nutritional value of meat

2.2.1 Chemical composition

Chemically meat is composed of four major components including water, protein, lipid, carbohydrate and many other minor components such as vitamins, enzymes, pigments and flavour compounds (Lambert et al., 1991). The relative proportions of all these constituents give meat its particular structure, texture, flavour, colour and nutritive value. However, because of its unique biological and
chemical nature, meat undergoes progressive deterioration from the time of slaughter until consumption (Lambert et al., 1991). Broadly, the composition of meat, after rigor mortis but before post-mortem degradative changes, can be approximated to 75% water, 19% protein, 3.5% soluble, non-protein, substances and 2.5% fat. The proteins in muscle can be broadly divided into those which are soluble in water or dilute salt solutions (the sarcoplasmic proteins), those which are soluble in concentrated salt solutions (the myofibrillar proteins) and those which are insoluble in the latter, at least at low temperature - the proteins of connective tissue and other formed structures (Lawrie and Ledward, 2006). The sarcoplasmic proteins are a mixture of several hundred molecular species. Several of the sarcoplasmic proteins are enzymes of the glycolytic pathway and may be present in more than one form (isozymes). Proteins of beef consist of essential amino acids such as leucine, isoleucine, lysine, methionine, cystine, phenylalanine, threonine, tryptophan, valine, arginine and histidine; of these the last two are considered essential for infants. Amino acids are important for maintenance and repair of body tissues in human (Lawrie and Ledward, 2006).

2.2.2 Nutritional value

The nutritive quality attributes of meat include the nutrient content, nutrient availability and caloric value (Adegoke and Falade, 2005). Meat is high in both protein quality and quantity. The nine essential amino acids that the body cannot make are found in meat, thus making it a complete protein, rich in most B complex, vitamins and some type of fat (Bastin, 2007).

Nutritional composition of red meats may vary somewhat according to breed, feed regime, season and meat cut, in general lean red meat has a relatively low fat content moderate in cholesterol and rich in protein, many essential vitamins and minerals (Williams et al., 2007).
2.2.2.1 Protein

Protein from meat origin is composed of 22 amino acids from which 10 essential and 12 non-essential (Smith, 2001). The essential amino acids must be supplied from dietary intake to meet the body's needs because the human body cannot synthesize them (Xiong, 2000). Muscle meat proteins are often classified based on their solubility into three groups; myofibrillar proteins, sarcoplasmic proteins and the stromal proteins. The myofibrillar proteins which are salt soluble proteins (1% salt concentration) mainly consist of actin and myosin (Barbut, 1995). Myosin plays an important role in fat emulsification and water holding capacity of products like sausages (Xiong, 2000).

2.2.2.2 Fat and fatty acids

Fat is an essential nutrient which supplies the body with energy and essential fatty acids and provides transport for fat soluble vitamins (A, D, E, K and carotenoids) (USDA/USD HHS, 2010). Properties that fat contributes to food products include shelf life, stability, physical state, flavor and aroma. Fatty acids are classified as saturated fatty acid (SFA), mono-unsaturated fatty acid (MUFA), and polyunsaturated fatty acids (PUFA). Animal fat containing high amounts of unsaturated fatty acids tends to be softer, this can be attributed to lipid oxidation (Enser et al., 2000).

2.2.2.3 Minerals

Minerals are one of the classes of essential nutrients in the human diet (Godber, 1994). The broad function of minerals is to help build body structure and to help coordinate body function (Martin and Coolidge, 1978). Meat is especially rich in iron, zinc, and phosphorus, however, lacks calcium, iodine, and magnesium in sufficient amounts (Romans et al., 2001). In meat, more than half of the iron is heme iron, the most readily absorbed form of iron (Romans et al., 2001). Heme iron primarily functions in transport of and in the binding of oxygen to hemoglobin.
in the blood. (Martin and Coolidge, 1978). Zinc is involved in numerous enzyme systems and is necessary for normal growth (Martin and Coolidge, 1978). Phosphorus has many functions throughout the body. It has a key role in maintaining the acid/base balance of blood, chemically reacts with macronutrients to release energy, is a component of ATP (functional form of energy), and is part of nucleoproteins that carry genetic information (Martin and Coolidge, 1978).

Animals require molybdenum, nickel, selenium, chromium, copper, fluorine, manganese, cobalt, magnesium, and iodine for cell functions; therefore, these minerals are present in beef muscle, but not in levels necessary for human nutrition (Romans et al., 2001).

### 2.2.2.4 Vitamins

Although meat is not a significant dietary source of most fat-soluble vitamins, it is a good source of many of the water soluble vitamins. Vitamins primarily function as cofactors in major metabolic pathways (e.g., TCA cycle, glycolysis, etc). Thiamin or Vitamin B-1 acts as a coenzyme and is essential for oxidation of glucose and, therefore, normal functioning of the gastrointestinal tract and nervous system (Martin and Coolidge, 1978). Beef is a good source of thiamin providing 4% of the recommend daily value per serving (85 g) (Romanset al., 2001). Riboflavin is involved in energy and protein metabolism and thus is essential for growth and development and mental vitality (Martin and Coolidge, 1978). Beef provide 13% of the recommended daily value per serving making them good dietary sources of riboflavin (Romans et al., 2001).

Niacin functions with enzymes that are principally involved in glycolysis, tissue respiration and fat synthesis (Martin and Coolidge, 1978). Meat provides a form of niacin that is more bio available than plant sources for humans (Romans, 2001). Beef is a good source of niacin providing 22% of the recommend daily
value per serving (Godber, 1994). Vitamin B-12 coenzymes are required for DNA synthesis and are necessary for normal function in cells of bone marrow, the nervous system and the gastrointestinal tract (Martin and Coolidge, 1978).

2.2.2.5 Carbohydrate content

Carbohydrate constitute less than one percent of the weight of meat, most of which is present as glycogen and lactic acid. Thus the liver is a good source of carbohydrate (Judge et al., 1990).

2.2.2.6 Pigments

The characteristic beef color is contributed by different meat pigments. Those pigments includes hemoglobin, myoglobin and cytochrome. Hemoglobin is present in the blood as an oxygen carrier from the lungs to the muscle cell or fiber and myoglobin (80 % to 90 % of the total pigment of meat) is the pigment present in muscle fiber which takes oxygen from the carrier hemoglobin. Cytochrome is associated with mitochondria and works in an electron transport chain (Aberle et al., 2001).

2.2.2.7 Enzymes

Meat contains protein splitting enzymes may be responsible for increasing the tenderness of meat during ripening or aging (Hiraie et al., 1973).

2.3 Meat quality

Meat quality is a term used to describe the overall meat characteristics including its physical, chemical, morphological, biochemical, microbial, sensory, technological, hygienic, nutritional and culinary properties (Ingr, 1989). Meat palatability depends on such quality attributes as aroma, flavor, color or appearance, tenderness and juiciness (Hiraie et al., 1973). Tshabalala et al. (2003) reported that consumer decision on the quality of meat is based on meat palatability components such as tenderness, juiciness and flavor. There is a
relationship between meat sensory characteristics and meat quality measurements such as pH, color and cooking losses (*Muchenje et al.*, 2008). Furthermore, quantifiable properties of meat such as water holding capacity, shear force, drip loss, cook loss, pH, shelf life, collagen content, protein solubility, cohesiveness, and fat binding capacity are indispensable for processors involved in the manufacture of value-added meat products (*Allen et al.*, 1998).

### 2.3.1 Colour


### 2.3.2 Flavour

Flavour is a complex sensation. It involves odor and taste. Of these, odor is the most important. Without it, one of the four primary taste sensations, bitter, sweet, sour or saline, predominates (*Lawrie*, 1991). Flavour and aroma are determined by many compounds such as hydrocarbons, aldehydes, ketones, alcohols, furans, triphenes, pyrols, pyridines, pyrazines, oxazols, thiazols, sulfurous, and others (*Calkins and Hodgen*, 2007).

### 2.3.3 Juiciness

Meat juiciness is one of the major parameters considered in the assessment of meat quality (*Muchenje et al.*, 2008). Meat juiciness is the wetness during first bite and sustained juiciness due to the fat in the meat (*Simela et al.*, 2005). The sensation of juiciness is closely related to the quantity and composition of intramuscular fat and the age of the animal (*Simela et al.*, 2005). The principal source of Juiciness in meat, as detected by the marbling that are present also serves enhance Juiciness during the cooking process when the melted fat apparently
become translated along the bands of perimysial connective tissue. This uniform
distribution of lipids throughout the muscle may act as barrier to moisture cooking
(Judge et al., 1990).

2.3.4 Tenderness

Tenderness appears to be the most important sensory characteristic of meat
and a predominant quality determinant (Sebsibe, 2006). Meat tenderness is rated
as the most important attribute of eating quality and is the factor that determines
the consumers continued interest in the meat (Simela et al., 2005). It is a function
of the collagen content, heat stability and the myofibrillar structure of muscle
(Muchenje et al., 2008).

2.3.5 Water holding capacity (WHC)

The water holding capacity (WHC) is the ability of meat to retain its water
or added water during application of external forces such as cutting, heating,
grinding or pressing (Lawrie, 1991).

NPPC (2002) defined water holding capacity (WHC) the ability of muscle
to retain naturally occurring moisture, and generally expressed as drip loss or
purge. Water holding capacity is important in meat processing because it affects
many of the physical properties of meat products, such as color, texture, juiciness
and tenderness. This ultimately will affect the overall product palatability (Brewer,
2004).

2.3.6 Firmness and texture

Firmness of flesh associated with pre and post mortem treatment of cattle
and may be connected with water holding capacity. Firmness does not seem to be
associated with fatness and well marble carcasses are unlikely to suffer from
watery muscle texture and hence coarse texture meat will be tougher eat (Copper
and Willis, 1984).
2.4 Deterioration of meat quality

A number of methods are employed throughout the meat industry to retard deteriorative changes and extend length of acceptability period. This depends mainly on preservative method and inherent properties of specific meat items. The post-mortem changes associated with conversion to meat subsequent storage and hand linking are caused by microorganism (bacteria, mold, and yeast), insect, indigenous enzymes naturally present in meat, exogenous enzymes (secreted by microorganism) and physical effects (freezer burn, drip, light fading and discoloration). The microbial sources include equipment clothing, and hands of personnel, air, water and doors (Judge et al., 1990).

2.4.1 Chemical deteriorative changes

Oxidative rancidity is the production of strong disagreeable odors and flavor due to exposure of fat to molecular oxygen in air. The chemical reactions that constitute the oxidative rancidity described by the presence of two molecular weight aldhydes, acids and ketone that formed during oxidation and decomposition of fatty acids molecules. The rate of auto-oxidation is enhanced by proxidants such as sodium chloride, some metal ions (e.g. copper, iron), heat ultraviolet, low PH, and numerous other substances or agents. Development of rancidity is retarded by avoidance of proxidants by storing meat in refrigerated darkness and minimizing amount of air in container (Judge et al., 1990).

2.4.2 Physical deteriorative changes

Dehydration is the loss of moisture from the meat surface that concentrated pigments and due to loss of intracellular water, reduces light reaction, the meat appear dark in color. Loss of moisture from the meat surface during storage produce dried state, course texture appearance that adversely affects eye appeal and acceptability. Several dehydration usually results in very dry products following cooking, and thus affects palatability. An excessive loss of moisture
from meat surfaces result in freezer burn which is characterized by cock-like texture and gray to tan color (Judge et al., 1990).

The loss weight that results is due to losses of meat moisture during refrigeration storage and known as it is shrinkage. Physical change accompanying shrinkage during prolonged refrigerated storage includes surface dehydration and discoloration (Judge et al., 1990).

Off-flavors may occur when meat stored in the presence of aromatic compound such as apples or onions due to high susceptibility of meat to the volatile materials (Judge et al., 1990).

2.5 Microbial ecology of meat

Microbial ecology has been defined as "the study of the interaction between the chemical, physical and structural attributes of a niche and the composition of its specific microbial population (Mossel and Struijk, 1992). The microbial ecology of meat is rarely viewed from this holistic aspect, but rather in terms of risk to health from pathogenic organisms and toxins, or risk to quality from microbial spoilage. The majority of this work has focused on bacteria. Whilst moulds and psychrotolerant (optimum range for growth 20-40°C, can grow at 0°C) yeasts are capable of growing on meat aerobically, causing black, white, blue-green and whisker spots, these are unable to complete with bacteria on fresh meat due to low growth rates and are therefore associated with salted, dried or frozen product. Their growth is completely inhibited under anoxic conditions (Marshall and Bala, 2001). In the healthy animal, tissues that ultimately become meat (muscle, fat and edible offal) are usually sterile (Mills et al., 2007).

Animal carcasses and meat cuts are easily contaminated with bacteria during the slaughter, dressing, cutting and packing process (Koutsoumanis and Sofos, 2004). If not properly handled, processed and preserved, meat will support the growth of these organisms thereby creating significant health risk. Sources of
contamination include faeces, ingesta, hide lymph nodes and intestines of the animals themselves, and air, water, soil, processing equipment, utensils and personnel from the abattoir environment (Lovatt, 2004). Despite ideal conditions existing on meat for the growth of many bacterial species, not all bacteria present initially multiply during storage. Meat invariably developed a characteristic flora whose composition can largely be predicted from knowledge of temperature, meat PH and gaseous atmosphere. It has been established that background organisms are antagonistic to pathogenic bacteria in anaerobically packed chilled meat, a process known microbial interference (Jay, 1996) further, it has been shown that a succession of different species leads to the eventual dominant microbiota on the final product (Jones, 2004).

2.6 Microbiology of meat

Meat being a good material for bacterial growth, its quality depends on the initial bacterial contamination. This contamination causes meat deterioration, lower quality, and some time illness may be caused by bacterial pathogens or their toxins (Jay, 2000). In live animals including cattle, microorganisms usually are present on the crotch, brisket and hind hocks region, and also in the gastrointestinal tract (Lim, 2002). In contrast, bacteria are normally absent in internal tissues, other than the gastrointestinal tract, due to immunological and non-immunological defense mechanisms (Roller, 2003). The population of microorganisms that contaminate meat is influenced by intrinsic microbiota of the animals and environmental conditions (Shapon and Shapon, 1994). Contamination initially occurs when pathogenic and spoilage microorganisms are transferred from the outer surface of the carcass to internal tissues during different slaughter processes (Fung, 2010). Microorganisms are also transferred through direct contact with the hide or indirectly through contact with workers’ hands or equipment used, and also via aerosols and dust generated from the hide during removal process (Huffman, 2002).
In addition, the water used for cleaning and sanitizing floors, instruments and containers also serve as the sources of contamination (Lim, 2002). A large variety of pathogenic microorganisms are commonly associated with carcass contamination, these include Clostridium perfringens, Staphylococcus aureus, Salmonella spp., E. coli, Campylobacter spp., Listeria monocytogenes and Yersinia enterolitica (Roller, 2003). Pathogenic E. coli such as E. coli O157:H7 and Salmonella spp. are the most frequently associated with fresh meat (Mead et al., 1999).

2.6.1 Escherichia coli

E. coli is gram negative, lactose fermenting, facultative aerobic short rod. First documented outbreak of E. coli food-borne gastroenteritis occurred in the U.S. in 1971 (Jay, 2000). The first outbreaks of food-borne hemorrhagic colitis in the U.S. was in 1982 (Jay, 2000). E. coli 0157:H7 was found to be the cause of two severe outbreaks characterized by hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS) (Rily et al., 1983). The first case of E. coli 0157:H7 infection in Italy was reported in 1988 (Mohmmed, 2013). E. coli 0157:H7 is one of the enterohemorrhagic E. coli (EHEC) serotypes that produce verocytotoxins (VTEC). These pathogen types were identified in 1977 and have been associated with several diseases in both humans and animals (Conedera et al., 1995). E. coli 0157:H7 is able to produce toxins which can cause very serious illness in humans, such as HC and HUS (Wang et al., 1999). The largest recorded food borne outbreak was associated with ground beef, and all raw meat should be considered a possible vehicle for hemorrhagic colitis (Jay, 2000). Escherichia coli O157:H7 is commonly found among the intestinal flora of cattle which are the primary reservoir (Harris and Savell, 2005).
2.6.2 Staphylococci aureus

*Staphylococcus aureus* is one of the most frequent pathogen that causes food-borne out breaks. It is responsible for staphylococcal food poison (SEP) by producing heat stable toxin (*Shapon and Shapon, 1994*). *Staphylococcus aureus* is a major pathogen for humans, ranging in severity from food poisoning or minor skin infections to severe life-threatening infection (*Jawetz et al., 2001*). Generally, *S. aureus* may be used as an indicator of general hygiene (*Roller, 2003*). Workers hands, equipment, and environmental conditions may harbor the bacterium (*Shapon and Shapon, 1994*).

2.6.3 Salmonella

Salmonella was first recognized in France by clinical pathologists in 1880. Named after D.E. Salmon, whose contemporary work led to the first isolation of *Bacillus cholerasuis*, otherwise known as *Salmonella enterica* (*Lim, 2002*). *Salmonella* are a member of the Enterobacteriaceae family and are Gram-negative, facultative anaerobic non-spore forming rods of 0.7-1.5 × 2-5 μm in dimension (*Harris and Savell, 2005*). *Salmonella* infection is spread among animals through the use of contaminated feed and the incidence tends to repack a peak where intensive stock raising is practiced (*Crossland, 1997*). *Salmonella* is commonly associated with raw or undercooked meat and egg products (*Huffman, 2002*). Chicken has the highest of positive samples among meat products; however, it is present in beef products as well (*Dureden et al., 1992*).

According to the Centers for Disease Control and Prevention (CDC), approximately 76 million cases of food borne illness occur each year in the United States, and approximately 14 million of these can be attributed to known pathogens (*Fung, 2010*). Food borne diseases are also to blame for approximately 325,000 hospitalizations and 5,000 deaths in the United States each year (*Fung, 2010*).
2.7 Biochemical changes in meat during post-mortem

In the living animal, aerobic metabolism is used to obtain energy. After slaughter, aerobic metabolism begins to fail due to the stored oxygen supply being depleted. After exsanguination, cessation of blood circulation shifts muscle metabolism from aerobic to anaerobic. It was reported that when muscle contracts in an anaerobic environment, glycogen disappears and lactic acid becomes the principal end product of glycolysis; whereas under aerobic conditions, lactic acid does not accumulate as it is oxidized to CO₂ and water (Mayes, 1993). One molecule of glucose will generate 3 moles of ATP via anaerobic glycolysis providing the high-energy phosphates necessary for post mortem (anaerobic) muscle contraction. Creatine phosphate is rapidly depleted as a result of postmortem metabolism, yet ATP may be maintained for several hours from anaerobic glycolysis. Accumulation of lactic acid in post-mortem muscle reduces the localized pH and muscle is converted to meat. Conversion of glycogen to lactic acid will continue to lower muscle pH until the glycogens (or ATP stores) are depleted or until the contractile proteins cease to function as a result of low intramuscular pH (Koochmarai, 1992).

The sequence of chemical steps by which glycogen is converted to lactic acid is essentially the same post-mortem as in vivo when the oxygen supply may become temporarily inadequate for the provision of energy in the muscle; but it proceeds further. Except when inanition or exercise immediately pre-slaughter has appreciably diminished the reserves of glycogen in muscle, the conversion of glycogen to lactic acid will continue until a pH is reached when the enzymes affecting the breakdown become inactivated (Lawrie and Ledward, 2006). In typical mammalian muscles this pH is about 5.4–5.5. An initial level of 600 mg glycogen/100 g muscle is required to attain this pH. Muscles which have an ultimate pH of 5.4–5.5 after post mortem glycolysis may still contain some residual glycogen, even though it is generally considered that there will be no
residual glycogen if the pH fails to fall to 5.4–5.5 during post-mortem glycolysis (Lawrie and Ledward, 2006).

The final pH attained, whether through lack of glycogen, inactivation of the glycolytic enzymes or because the glycogen is insensitive (or inaccessible) to attack, is referred to as the ultimate pH; this is generally about 5.5, which is the iso-electric point of many muscle proteins (Immonen et al., 2000). Both the rate and the extent of the post-mortem pH fall are influenced by intrinsic factors such as species, the type of muscle and variability between animals; and by extrinsic factors such as the administration of drugs pre-slaughter and the environmental temperature; exercise pre-slaughter is also a known factor which produces dry firm dark (DFD) meat which has a pH of around 7.0 (Shimada et al., 2004).

2.8 Causes of meat spoilage

Pre-slaughter handling of livestock and post-slaughter handling of meat play an important part in deterioration of meat quality. The glycogen content of animal muscles is reduced when the animal is exposed to pre-slaughter stress which changes the pH of the meat, to higher or lower levels, depending on the production level of lactic acid (Miller, 2002). Lactic acid is produced due to the breakdown of glycogen content of animal muscles via an anaerobic glycolytic pathway (Rahman, 1999). Higher levels of pH (6.4–6.8) result in Dark, Firm and Dry (DFD) meat. Long term stress causes DFD meat which has a shorter shelf life (Miller, 2002). Sever short term stress results in a Pale, Soft and Exudative (PSE) meat. PSE meat has a pH lower than normal ultimate value of 6.2 which is responsible for the breakdown of proteins, providing a favorable medium for the growth of bacteria (Chambers and Grandin, 2001).

There are three main mechanisms for meat and meat products spoilage after slaughtering and during processing and storage. (a) microbial spoilage, (b) lipid oxidation and (c) autolytic enzymatic spoilage.
2.8.1 Microbial spoilage

Meat and meat products provide excellent growth media for a variety of microflora (bacteria, yeasts and molds) some of which are pathogens (Jay et al., 2005). The intestinal tract and the skin of the animal are the main sources of these microorganisms. The composition of microflora in meat depends on various factors. (a) pre-slaughter husbandry practices (free range Vs intensive rearing), (b) age of the animal at the time of slaughtering, (c) handling during slaughtering, evisceration and processing, (d) temperature controls during slaughtering, processing and distribution (e) preservation methods, (f) type of packaging and (g) handling and storage by consumer (Cerveny et al., 2009). Mold species include Cladosporium, Sporotrichum, Geotrichum, Penicillium and Mucor while yeasts species include Candida spp., Cryptococcus spp. and Rhodotorula spp. (Garcia-Lopez et al., 1998). Bacteria species include Pseudomonas, Micrococcus, Streptococcus, Sarcina, Lactobacillus, Salmonella, Escherichia, Clostridium and Bacillus (Lin et al., 2004).

Hayes et al. (2003) found Enterococcus spp. to be the most dominant bacteria on 971 of the 981 samples (99%) of all meat (chicken, turkey, pork and beef) in the state of Iowa. About 97% of pork samples contained Enterococci with 54% of isolates identified as Enterococcus faecalis and 38% as Enterococcus faecium, 3.4% as Enterococcus hirae, 2.4% as Enterococcus durans, 0.8% as Enterococcus

Garcia-Lopez et al. (1998) reported that the growth of Enterobacteriaceae and Pseudomonas were more prevalent on modified atmosphere packed meat (especially on pork) than on vacuum packed meat, their growth being favoured by storage at 5°C. Sentence (1991) reported that Pseudomonas spp. growth rate was considerably slow at 0°C, but increased at 2°C and affected the shelf life of meat. He also noticed slow Salmonella growth below 7°C, which increased above 7°C and affected the shelf life of meat. Borch et al. (1996) reported that the growth of lactic acid bacteria on bologna-type sausage was retarded 2 and 4 fold with
decreases in temperature from 7-2°C and from 7-0.6°C, respectively. **Russell et al. (1996)** stated that a favorable pH for the growth of spoilage bacteria for meat is in the range of 5.5-7.0. Slime formation, structural components degradation, off odors and appearance change were found in meat as a result of microbial growth within this pH range. The methylamine, dimethylamine and trimethylamine have been commonly detected during bacterial spoilage by **Garcia-Lopez et al. (1998)**.

### 2.8.2 Lipid oxidation

Autoxidation of lipids and the production of free radicals are natural processes which affect fatty acids and lead to oxidative deterioration of meat and off-flavours development (**Simitzis and Deligeorgis, 2010**).

After slaughtering of animals, the fatty acids in tissues undergo oxidation when the blood circulation stops and metabolic processes are blocked (**Linares et al., 2007**). Lipid oxidation is the reaction of oxygen with double bonds of fatty acids (**Hultin, 1994**). It involves three stage free radical mechanisms: Initiation, propagation and termination (**Fernindezet al., 1997**). Oxidation of lipids in meat depends on several factors including fatty acid composition, the level of the antioxidant vitamin E (tocopherol) and prooxidants such as the free iron presence in muscles. Polysaturated fatty acids are more susceptible to lipid oxidation.

Hydro-peroxides are produced due to the lipid oxidation of highly unsaturated fatty acid fractions of membrane phospholipids, which are susceptible to further oxidation/ decomposition (**Simitzis and Deligeorgis, 2010**). In meat, lipid hydrolysis can take place enzymatically or non-enzymatically. The enzymatic hydrolysis of fats is termed lipolysis or fat deterioration and is governed by specific enzymes such as lipases, esterases and phospholipase. Lipolytic enzymes could either be endogenous of the food product (such as milk) or derived from psychrotrophic microorganisms (**Ghalyet et al., 2010**).
2.8.3 Autolytic enzymatic spoilage

Enzymatic actions are natural process in the muscle cells of the animals after they have been slaughtered and are the leading cause of meat deterioration. The enzymes have the ability to combine chemically with other organic compounds and work as catalysts for chemical reactions that finally end up in meat self deterioration (Tauro et al., 1986). In the autolysis process, the complex compounds (carbohydrates, fats and protein) of the tissues are broken down into simpler ones resulting in softening and greenish discoloration of the meat. These autolysis changes include proteolysis and fat hydrolysis which are prerequisite for microbial decomposition. Excessive autolysis is termed “souring” (Tauro et al., 1986).

Post-mortem breakdown of polypeptides are the result of tissue proteases and is responsible for flavor and in textural changes in meat (Toldra and Flores, 2000). Post mortem aging of red meat results in the tenderization process (Huss, 1995). Post-mortem autolysis takes place in all animal tissues but at different rates in different organs, quicker in glandular tissue such as the liver and slower in striated muscle (Fearon and Foster, 1922). The enzymes calpains, cathepsins and aminopeptidases are found to be responsible for the post mortem autolysis of meat through digestion of the z-line proteins of the myofibril (O’Halloran et al., 1997). Among these enzymes, calpains has been described as a preliminary contributor to the proteolytic tenderization process of meat. Cathepsins were, also, found to contribute to tenderization at low pH. Proteolytic enzymes are active at low temperatures (5°C) which lead to deterioration of meat quality due to growth of microbes and biogenic amines production (Kuwahara and Osako, 2003).
2.9 Preservation of Meat

Meat preservation became necessary for transporting meat for long distances without spoiling of texture, colour and nutritional value after the development and rapid growth of super markets (Nychas et al., 2008). The aims of preservation methods are. (a) to inhibit the microbial spoilage and (b) to minimize the oxidation and enzymatic spoilage.

Traditional methods of meat preservation such as drying, smoking, brining, fermentation, refrigeration and canning have been replaced by new preservation techniques such as chemical, biopreservative and nonthermal techniques (Zhou et al., 2010). Current meat preservation methods are broadly categorized into three methods (a) controlling temperature (b) controlling water activity (c) use of chemical or biopreservatives (Zhou et al., 2010). A combination of these preservation techniques can be used to diminish the process of spoilage (Bagamboula et al., 2004).

2.9.1 Low temperature methods

The basic aim of cooling techniques is to slow or limit the spoilage rate as temperature below the optimal range can inhibit the microbial growth (Cassens, 1994). Low temperature methods of storage are used in three levels. (a) chilling (b) freezing and (c) super chilling. All these levels help to inhibit or completely stop bacterial growth (Zhou et al., 2010). However, the growth of psychrophilic group of bacteria, yeasts and molds is not prevented by all levels of refrigeration (Neumeyer et al., 1997) and both enzymatic and non enzymatic changes will continue at a much slower rate (Berkel et al., 2004).

2.9.1.1 Chilling

Chilling is employed at slaughtering plants immediately after slaughtering and during transport and storage. It is necessary to reduce the temperature of carcass immediately after evisceration to 4°C within 4 h of slaughtering (USDC,
Chilling is critical for meat hygiene, safety, shelf life, appearance and nutritional quality (Zhou et al., 2010).

2.9.1.2 Freezing

Freezing is an excellent method of keeping the original characteristics of fresh meat. Meat contains about 50-75% by weight water, depending on the species, and the process of freezing converts most of water into ice (Heinz and Hautzinger, 2007).

2.9.1.3 Super chilling

Super chilling is a different concept than refrigeration and freezing and it has the potential to reduce storage and transport costs (Reynolds, 2007).

2.9.2 Controlled water activity methods

Microbiological safety of food is directly influenced by the water activity (a\textsubscript{w}). The term water activity (a\textsubscript{w}) refers to water which is not bound to food molecules and can support the growth of microorganisms. It represents the ratio of the water vapour pressure of the food to the water vapour pressure of pure water under the same conditions (Ghaly et al., 2010). Water activity in meat products is equivalent to the relative humidity of air in equilibrium with the product (Comaposada et al., 2000).

2.9.2.1 Sodium chloride

Borch et al. (1996) stated that salt-sensitive microorganisms, such as Pseudomonas spp. and Enterobacteriaceae, did not grow in meat when the water activity (a\textsubscript{w}) was reduced from 0.99 to 0.97 with the addition of 4% sodium chloride.
2.9.2.2 Sugars

Chirife (1994) reported that sucrose restrained the growth of \textit{Staphylococcus aureus} by lowering water activity.

2.10 Chemical methods for controlling microbial spoilage

Energy intensive freezing operations are the greatest way to preserve carcass, meat and meat products for a longer time which inhibits bacterial growth, but not the psychrophiles and the spores. Most of these survive freezing and grow during thawing (Neumeyer \textit{et al.}, 1997). Traditional methods for preservation of meat by salting and pickling are well accepted procedures. Other chemicals have been used as food additives for preservation of meat but every country has drawn its rules and regulations and established limits for the purpose of prevention of harmful effects to human (Cassens, 1994).

2.10.1 Sodium chloride

It inhibits microbial growth by increasing osmotic pressure as well as decreasing the water activity in the micro-environment. Some bacteria can be inhibited by concentrations as low as 2 \% (Urbain, 1971).

2.10.2 Nitrites

The nitrites used in meat preservation industry are always in the form of salts such as sodium nitrite or potassium nitrite. Nitrites provide stabilized red meat color, cured meat flavor and rancidity retardation (Jayet \textit{et al.}, 2005).

2.10.3 Lactic acid

Lactic acid has shown antimicrobial activities against many pathogenic organisms such as \textit{Clostridium botulinum} because of its abilities to reduce pH level, exert feedback inhibition and interfere with proton transfer across cell membranes (Doores, 2005).
2.10.4 Ascorbic acid

Ascorbic acid (vitamin C) the enhanced activities include both the antioxidant properties and the sequestering of iron (Tompkin et al., 2007).

2.10.5 Benzoic acid

The benzoic acid is generally used to inhibit yeasts and fungi rather than bacteria (Feiner, 2006).

2.10.6 Sorbic acid

Sorbic acid (2, 4-hexadienoic) and its salts are widely used throughout the world as meat preservatives for inhibiting bacteria and fungi (Davison et al., 2005).

2.11 Chemical methods for controlling oxidative spoilage

Freeze storage cannot prevent oxidative spoilage and microbial/enzymatic spoilage (Jay et al., 2005). Thus, chemical preservation methods are quite beneficial in combination with refrigeration in order to optimize stability, product quality while maintain freshness and nutritional value (Cassens, 1994). The primary antioxidants act as a radical scavengers or hydrogen donors or chain reaction breakers while the secondary act as peroxide decomposers (Andre et al., 2010).

2.11.1 Phosphates

Among the antioxidants in food additives, phosphates were one of the first investigated for their potential antioxidant activities in meat products (Trout and Dale 1990).

2.11.2 Salts

According to the Canadian Food and Drug Act, salts of sodium and potassium are Good Manufacturing Practice-listed with meats (DJC, 2009).
2.11.3 Acids

The pH plays an important role on enzymatic activities and that depends on type of acid used. Rosell and Toldra (1996) reported that the addition of ascorbic acid inhibits enzymatic (m-calpain) activity by 40-45%.

2.12 Meat emulsion

An emulsion consist of two immiscible liquids where one of the liquids is the dispersed as fine droplet (dispersed phase) within the other liquid which forms the continuous phase (McClements, 2005). According, to this definition meat batters are not a true emulsion since this don’t contain two liquid phases. However, chopped meat mixture is generally referred to as a "meat emulsion" because the suspension of fat globules dispersed within a continuous protein and water network suggests a similar structure to that of an emulsion. A meat emulsion is a multiphase system formed by the comminution of meat, fat, salt, and other ingredients (Varnam and Sutherland, 1995). Meat products such as frankfurters and bologna are example of meat emulsion. In an emulsion, proteins are present in three different phases. the protein matrix, the aqueous phase and the interfacial film (IPF) around fat globules (Gordon et al., 1992). Meat proteins represent the major functional ingredients which serve as the natural emulsifying agent in a meat emulsion (Alvarez et al. 2007). The stability of a meat emulsion is affected by both the type and amount of protein in these phases (Gordon et al., 1992).

2.12.1 Meat emulsion theory

The stability of a meat emulsion is explained by two theories. the emulsion theory and the physical entrapment theory. The emulsion theory proposes the formation of an interfacial protein film (IPF) around the fat globules in the meat emulsion. Myosin is the major protein that contributes to the mass of IPF. The emulsifying property of myosin plays a key role in stabilization of the emulsion.
(Varnam and Sutherland, 1995). Addition of salt during the chopping of meat, changes the conformational structure of myofibrillar protein molecules by increasing the hydrophobicity of their surface (Voutsinas et al., 1983). The heavy mereomyosin molecules, subunits of myosin having high hydrophobic surface area, orient towards the fat globules and light mereomyosin being hydrophilic, faces the aqueous phase. This result in the formation of a covering layer of myosin around the fat globules (Jones, 1984). During cooking, the IPF undergoes changing during which IPF is penetrated by small pores and the exudation fat from these pores maintains the integrity of the IPF (Varnam and Sutherland, 1995).

2.13 Storage of meat

Meat one of the perishable foods containing good culture media i.e. high in moisture, nearly neutral pH and high in nutrient. For these reasons, the PH and microbial contamination must be controlled. The change in PH during freezing and subsequent storage might be caused by the increase in concentration of the soluble material in liquid phase as a direct consequence of ice formation by the subsequent precipitation and probably the interaction of proteins with ionic substances in the unfrozen food (Mohammed, 2005). Precipitation of salts would appear to be the cause of large PH change (Judge et al., 1990), mentioned that the initial microbial load of meat result from introduction of microbiological contamination in to vascular system or subsequent contamination that occur on meat surface during slaughtering, cutting, processing, storage and handling of meat.

2.14 Consumption of processed meats – what is the issue

The variation in processed meat products on the market is huge. In general the term processed meat or cured meat refers to nitrite preserved, salted and in many cases also smoked meat (Santarelli et al., 2008). Common processed meat products include bacon, ham, sausages (cooked sausages), salamis (raw sausages, fermented and dried). The majority of the processed meat products available and
consumed in Denmark are produced from red meat. Red meat is in most cases and in the present work defined as meat of four legged animals. Meat from these animals has a higher content of myoglobin and therefore also appears redder than e.g. poultry and turkeys which is referred to as white meat (Santarelli et al., 2008).

Curing is basically the addition of sodium chloride and nitrite or nitrate to meat. As a general rule the EU legislation allows the addition of 150 mg nitrite per kg meat whereas the Danish National Provisions allows the addition of 60 mg nitrite per kg meat (Santarelli et al., 2008).

2.14.1 Health concerns

Several epidemiologic studies indicate associations between consumption of red and processed meat and increased risk of e.g. colorectal cancer (Santarelli et al., 2008), stomach cancer (Larsson et al., 2006), pancreatic cancer (Larsson and Wolk, 2012) but also with increased risk of cardiovascular diseases and other causes of death (Rohrmann et al., 2013).

2.15 Meat processing

Meat production commences with transport of the animals to the facility, where they are stored in "lairage" pens, and washed rigorously, either by swimming or high velocity jets, prior to slaughter. The animals are pre-stunned prior to slaughter by spanning the head with metal tongs and passing an electrical current through the brain inducing an epileptic seizure. This "head only" stun does not, however, stop the heart and so actual slaughter is achieved by severing the throat prior to recovery, cutting the carotid artery, jugular veins, trachea, oesophagus and various, and the carcass hung so that blood drains from the severed vessels in a manner consistent with religious (halal) slaughter (Grandin, 2001). Muscle does not terminate all functions at the time of death. Glycolysis continues, but oxidative phosphorylation cannot (due to the lack an oxygen transport system, therefore the
pyruvate generated is converted to lactic acid, which accumulates in the muscle. This persists until the majority of glycogen stores are also depleted, and the PH of the muscle falls from 7.0 to around 5.5, achieving a final "ultimate PH" when the process of rigor mortis (latin- "stiffness of death") has completed (Greaser, 2001). This stage of chemical change is known as conditioning and is influenced by muscle type, temperature, time, cutting and hanging of the carcass, typically taking around 16 hours for lamb (Honikel, 2004).

Rigor-mortis itself is a consequence of the formation of permanent cross-linkages between the muscle filaments, due to loss of regenerated ATP as glycolysis ceases (Aberle et al., 2001). The process is gradual, as not all possible cross bridges form at the same time. The overall shortening resulting from rigor mortis is therefore small (10-15) and the resulting toughness can be reversed during chilled storage (aging) through the action of endogenous proteolytic enzymes (e.g calpain) present in the meat (Millsetal., 2007).

2.16 Processed meat products

Those are products in which properties of fresh meat have been modified using one or more procedures such as alteration of colour, grinding or chopping, addition of seasoning or heat treatment. The original purpose of meat processing was preservation by inhibiting microbial decomposition as well as processing that result in flavorful and nutritious products. Increased price for lean meat has also altering processing practices and has encouraged the incorporation of increased percentage of less expensive fat (Judge etal., 1990). The processed products should be uniform in colour, texture, and fat distribution and suitable to conveniently and cut into portion size with minimum of waste to consumer. Also reduced cooking loss improved tenderness and texture and increased shelf life are some of most important characteristics of processed meat (Price and Schweigert, 1987).
Comminuted products are those made from raw meat material that has been reduced into small meat pieces, chips, or flaks. Some comminuted products can be classified as sausage and other are not. Two main advantages are gained from all comminuted products, i.e. improved uniformity of product to more uniform particle size, distribution of ingredients and increased in tenderness, as meat is subdivided into smaller particles. Equipment commonly used for comminution includes the meat grinder, bowl chopper, emulsion mill and flaking machines. Grinders are usually employed for the first step in subdivided into smaller particles. Equipment commonly used for comminution of meat and some many commercial products (Judge et al., 1990). Many commercial products being as ground meat, chunks flakes, slices or fillets that are formed into roast, steak, patties or nuggets. These often are marketed as burger steaks, and many even be breaded and precooked (Judge et al., 1990). The original for meat processing was preservation by inhibiting or suppressing microbial decomposition. In addition to preventing spoilage, preservation also results in flavorful and nutritious products. Meat processing has additional aspects of providing both convenience and variety (Kramlich et al., 1982).

Processed meat means a meat product containing no less than 300g/kg meat, where either singly or in combination with other ingredients or additive, has undergone of processing other than deboning, slicing, mincing or freezing or includes manufactured meat and cured and / or dried meat flesh in whole cuts or pieces. Meat was processed as early as prehistoric times, probably drying in the sun and later by smoking over wood. Today, meat is processed with salt, color fixing ingredients, and seasonings in order to import desired palatability traits to intact and comminuted meat products. Intact meat product includes bacon, corned beef, smoked and pork hock. Comminuted meat products include all types sausage item (NIIR, 2004).
2.17 Some types of meat products

2.17.1 Sausages

The term sausage is derived from the Latin word “salsus” meaning salt, referring to salted, seasoned, chopped meat product. They are usually cylindrical in shape, mainly because sausage mixtures have traditionally been encased in animal intestines or stomachs (Pearson and Gillett, 1996).

2.17.2 Frankfurter

Frankfurters are emulsion type cooked sausages which are very popular and highly consumed meat product in many countries (Özvural and Vural, 2008). Frankfurters which are commonly referred as “Wieners” or “Hotdogs” are usually made from beef or pork or a combination and are flavored with spices and smoke application (Gonzalez-Vinas et al., 2004). The word frankfurter originated from Frankfurt, Germany, where pork sausages originated (National Hotdog and Sausage Council, 2010).

2.18 Meat safety

Safety is a primary concern of the consumer when purchasing meat. Customers regard "use by" dates as a guaranteed date of safety. Therefore, the manufacturer must be certain the shelf-life recommendation is correct (Becker, 2002). Consumer safety issues associated with red meats are not restricted to microbiological hazards. Chemical hazards include presence of pesticides, hormones, antibiotics, and chemicals from production and processing. Physical hazards are typically foreign bodies such as glass, metal and plastic. Steps to eliminate these hazards must be considered as part of the manufacturers HACCP programme but don’t impact significantly on shelf-life (Fungeetal., 2001). The catalogue of pathogenic bacteria that have been found on red meat is extensive. However, only some of these bacteria are implicated on food – poisoning disease, notably *Salmonella*, *Escherichia coli* 0157:H7, *Campylobacter*, *Clostridium*
botulinum, Aeromonas hydrophila, Yersinia enterocolitica in pork, and Listeria monocytogenes in processed meats (Funge et al., 2001).

All of these pathogens are considered adulterants in high risk "ready to eat" foods (that require no additional cooking). Despite the fact that raw, fresh meat will be subjected to cooking as a final hygienic control step prior to consumption; many countries have now adopted a "zero tolerance" approach to the presence of some pathogenic microorganism in the USA, a well-publicised outbreak associated with under cooked hamburger.(501 recorded cases of food poisoning, 151 hospitalizations , 45 cases of haemolytic uraemic disease (HUD) and 4 deaths), lead to the causative organism Escherichia coli 0157.H7 being declared an adulterant of meat. A large-scale surveillance programme was established to monitor all beef destined for the US burger market, including most New Zealand (NZFSA,2006). The hygienic objectives of post-slaughter carcass and product management are, therefore, to first minimize contamination. As total eradication is impractical, the second objective is to restrict subsequent proliferation (Bell et al., 1998). This second objective is of paramount importance when considering technologies that extend shelf-life. Most of the meat- borne pathogens described above (Salmonella, Escherichia coli0157.H7 and Campylobacter) are mesophilic. Whilst there is significant risk of these organisms proliferating (with associated hazard to health) at ambient temperatures in the "wet market" scenario, they will not multiply during chilled or frozen storage. Refrigeration therefore provides a simple technology to safety extend shelf-life (Bell,2001).

Whilst non-proteolytic clostridium botulinum, Aeromonas hydrophila, Yersinia enterocolitica and listeriamonocytogenes, can grow at lower temperatures (Cl. Botulinum cannot produce toxin below 2-3C, minimum growth temperature for the remainder is 0-4C), growth can be limited by low substrate PH and storage atmosphere. Generally, the minimum temperature for growth increases as CO2
concentration increases and PH falls, thus storage life can be extended by incorporating CO2 into the package product.

2.19 Agashi as meat product.

Agashi was introduced to Sudan by the Hausa and Alvlata tribes came from regions of Central Africa, particularly Nigeria. Agashi is an African word means bar-becue meat in which different types of spices are added as known in West Africa. Agashi started entering to Sudan in the 1960s and was confined to specific areas. However, in early 1990, it began to spread in most of States of Sudan. Nowadays Agashi is one of the most popular snack foods and its preparation is still limited to certain tribes (Sulieman et al., 2012).
CHAPTER THREE

3. MATERIALS AND METHODS

3.1 Materials and Methods

Six cooked Agashi samples were collected after processing in sterilized containers from three different locations in Khartoum State (Khartoum, Khartoum North, Omdurman). Triple samples were taken from open areas (streets, transportation places) and closed areas (big restaurants, modern restaurants) in each location. Samples were transported under aseptic conditions to the microbiology laboratory and chemistry laboratory in Khartoum University, Shambat.

3.2 Chemical analysis

3.2.1 Moisture content

The moisture content was determined according to standard methods of association of official analytical chemists (AOAC, 2003).

Principle

The moisture content is a weighed sample removed by heating the sample in an oven under atmospheric pressure at 105 ±1°C. Then the difference in weight before and after drying is calculated as a percentage from the initial weight.

Procedure

A sample of 5g ±1mg was weighed into a pre-dried and tarred dish. Then the sample was placed into an oven (NO.03-822, fn400, turkey) at 105±1°C until a constant weight was obtained. After that the covered sample was transferred to desiccators and cooled to room temperature before reweighing. Triplicate results were obtained for each sample and the mean value was reported.
Calculation

\[
\text{Moisture content \%} = \frac{(M_2 - M_3)}{(M_2 - M_1)} \times 100
\]

Where:

\(M_1\) = weighed dish + cover

\(M_2\) = weight of dish + cover + sample before drying

\(M_3\) = weight of dish + cover + sample after drying

The dry matter (DM) percentage was calculated by subtracting the percentage of moisture from 100%.

3.2.2 Fat content

The crude fat in the product was determined according to the standard method of AOAC (2003).

Principle

The method determines the substances which are soluble in hexane (B.P, 40 – 60ºC) and extractable under the specific conditions of Soxhlet extraction method. The dried hexane extract is weighed and reported as percentage of the dry matter as crude fat.

Procedure

A sample of 5g ±1mg was weighed into an extraction thimbles (30-100 mm) and covered with cotton that previously extracted with hexane. Then, the sample and a pre-dried and weighed erlenmeyer flask containing about 150 ml hexane (No1622, BDH, England) were attached to the extraction unit (Electrothermal, England) and the temperature was adjusted to produce about 150 to 200 drops of the condensed solvent per minute for 16 hours. At the end of the distillation period, the flask with was disconnected from the unit and the solvent was redistilled.
Later, the flask with the remaining crude hexane was put in an oven at 105°C for 3 hours, cooled to room temperature in a desiccators, reweighed and the dried extract was registered as crude fat (% DM) according to the following formula:

\[
\text{Fat content (\%)} = \frac{(W_1 - W_2)}{W_3} \times 100
\]

Where:

- \(W_1\) = weight of flask and ether extract
- \(W_2\) = weight of empty flask
- \(W_3\) = initial weight of sample

### 3.2.3 Crude protein

The crude protein was determined in all samples by micro-kjeldahl method using a copper sulphate and sodium sulphate catalyst according to the official methods of AOAC (2003).

**Principle**

The method consists of sample oxidation and conversion of nitrogen to ammonia, which reacts with the excess amount of sulphuric acid forming ammonium sulphate. The solution is made alkaline and the ammonia is distilled into a standard solution of boric acid (2%) to form the ammonia–boric acid complex, which is titrated against a standard solution of HCL (0.1). Accordingly, the crude protein content is calculated by multiplying the total N% by 6.25 as a conversion factor for protein.

**Procedure**

Two gm ± 1mg sample was accurately weighed and transferred together with 2-3 glass pellets, kjeldahl catalyst (No33064, BDH, Germany) and 30 ml
concentrated sulphuric acid into kjeldahi digestion flask. After that, the flask was placed into a kjeldahl unit (Tecator, Sweden) for about 3 hours, until a colorless digest was obtained. Following, the flask was left to cool to room temperature. The distillation of ammonia was carried out in 30ml boric acid (2%) by using 40ml distilled water and 60 ml sodium hydroxide solution (33%). Finally, the distillate was titrated with standard solution of 0.1HCL in the presence of 2-3 drops of indicator (Bromocresol green and methyl red) until a brown reddish colour was observed.

**Calculation**

Crude protein% = \( \frac{(TV \times N \times 14.00 \times F) \times 100\%}{1000 \times \text{sample weight (g)}} \)

Where:

TV = actual volume of HCL used for sample

N = normality of HCL

F = protein conversion factor = 6.25

**3.2.4 Ash content**

The standard analytical methods of AOAC (2003) were used for determination of ash content in the samples.

**Principle**

The inorganic materials which are varying in concentration and composition are customary determined as a residue after being ignited at a specified heat degree.
**Procedure**

A sample of 2gm ±1mg was weighed into a pre-heated, cooled weighed and tarred porcelain crucible and placed into a muffle furnace (Carbolite, Sheffield, England) at 550 to 600°C until a constant weight and a white gray ash was obtained. The crucible was transferred to a descanter then allowed to cool to room temperature and weighed. The ash content was calculated as a percentage based on the initial weight of sample.

**Calculation**

\[
\text{Ash \%} = \frac{\text{(Wt of crucible + ash)} - \text{(Wt of empty crucible)}}{\text{Initial weight (Wt)}} \times 100
\]

**3.2.5 Crude fibre content**

The crude fibre was determined according to the official method of the AOAC (2003).

**Principle:**

The crude fibre is determined gravimetrically after the sample is being chemically digested in chemical solutions. The weight of the residue after ignition is then corrected for ash content and is considered as a crude fibre.

**Procedure:**

About 2gm ± 1 mg of a defatted sample was placed into a conical flask containing 200 ml of H₂SO₄ (0.26 N). The flask was then, fitted to a condenser and allowed to boil for 30 minutes. At the end of the digestion period, the flask was removed and the digest was filtered (under vacuum) through a porcelain filter crucible (No.3). After that, the precipitate was repeatedly rinsed with distilled boiled water followed by boiling in 200 ml NaOH (0.23 N) solution for 30 minutes under reflux condenser and the precipitate was filtered, rinsed with
hot distilled water, 20ml ethyl alcohol (96%) and 20 ml diethyl ether.

Finally, the crucible was dried at 105 °C (overnight) to a constant weight, cooled, weighed, ashed in a Muffle furnace (No.20. 301870, Carbolite, England) at 550-600 °C until a constant weight was obtained and the difference in weight was considered as crude fiber.

**Calculation:**

\[
\text{Crude fibre (\%) = } \frac{(W_1 - W_2)}{\text{Sample weight (gm)}} \times 100\%
\]

Where:

- \( W_1 \) = weight of sample before ignition (gm).
- \( W_2 \) = weight of sample after ignition (gm).

**3.2.6 Carbohydrate content**

Carbohydrate content was calculated by difference according to the following equation:

Total carbohydrates = 100% - (Moisture + Protein + Fat + Ash).

**3.3 Microbiological analysis**

**3.3.1 Preparation of serial dilutions**

Ten grams of each sample were weighed aseptically and homogenized in 90 ml of sterile diluent (0.1% peptone water to give \(10^{-1}\) dilution. Aseptically 1ml from the dilution. \(10^{-1}\) was transferred to a tube containing 9ml sterile diluents. This makes a dilution of \(10^{-2}\) then in the same way the preparation of serial dilutions was continued up to the \(10^{-6}\) (Harrigan, 1998).
3.3. 2 Total bacterial count

Total viable count of bacteria was carried out by using the pour plate count method as described by Harrigan (1998). One ml of every dilution was transferred aseptically into sterile plate and to each plate 10 - 15 ml of sterile melted and cooled (42°C) plate count agar were added. The inoculums was well mixed with medium and allowed to solidify. The plates were then incubated at 37°C for 48 hours. A colony counter was used to count the viable bacteria and the results were presented as cfu/g.

3.3.3 Determination of coliform bacteria

It was carried out by using the most probable number (MPN) technique (Harrigan, 1998).

3.3.4 Presumptive test for coliforms

One ml of each of three first dilutions \( 10^{-1}, 10^{-2}, \text{ and } 10^{-3} \) was inoculated in triplicates of 9ml of MacConkey broth in test tubes containes Durham tubes. The tubes were incubated at 37°C for 48 hour. The production of acid together with sufficient gas to fill the concave of the Durham tubes is recorded as positive presumptive test (Harrigan, 1998).

3.3.5 Confirmed test for coliforms

From every tube showing positive result, a tube of Brilliant Green(2%) BileBroth was inoculated by using sterile loop. The tubes were incubated at 44°C for 48 hours. Then the tubes showing positive and negative result were recorded. The most probable number of total coliform was found out by using the most probable Number(MPN) Tables (Harrigan, 1998).

3.3.6 Test for E.coli

Medium used EC Broth. From every tube showing positive result in the presumptive test was used incubate a tube of EC Broth containing durham tubes.
The tubes were incubated at 44.5°C for 24 hours. Tubes showing any amount of gas were considered positive. Then the most probable number (MPN) was recorded. For farther confirmation of *E. coli* tubes of EC showing positive result at 44.5°C for 24 hours were streaked on (EMB) agar Eosin Methyleane Blue agar plates. The plates were incubated at 44°C for 48 hours. Colonies of *E. coli* are usually small with metallic green sheen on EMB agar (Harrigan, 1998).

**3.3.7 Staphylococcus aureus**

Amount of 0.1 ml from count dilution was transferred onto surface of sterile well solidified Baird Parker agar medium in plates, and spread all over the plates using sterile bent glass rod. Then incubated for 24-36 hours at 37°C and the plates were examined for *Staphylococcus aureus* which appeared as black shiny convex colonies surrounded by a clear zone of 2-5m in width (Harrigan, 1998).

**3.3.8 Detection of Salmonella**

Twenty five grams of sample were weighed aseptically and mixed well with 250 ml sterile nutrient broth. These were incubated at 37°C for 24 hours. Then 10 ml were drawn aseptically and added to 100 ml of selenite cystine broth. The broth was incubated at 37°C for 24 hours. Then with a loop full streaking was done on solidified bismuth sulphite agar in plates were then incubated at 37°C for 72 hours. Black metallic sheen colonies indicated the presence of *Salmonella* italies. A confirmatory test was carried out by taking a discrete black sheen colony and sub culturing it in triple sugar iron agar tubes. Production of a black colour at bottom of the tube confirmed the presence of *Salmonella* (Harrigan, 1998).

**3.4 Sensory evaluation**

Sensory evaluation was done as described by Ranganna (2001). Using the hedonic scoring test method. In this method 20 trained panelists from the Food Science and Technology Dept., College of Agricultural Studies, Sudan University
of Science and Technology were asked to evaluate the products with regard of their colour, flavour, taste, overall acceptability, using the following hedonic scale:

1= excellent, 2= very good, 3= good, 4= acceptable, 5= unacceptable.

3.5 Statistical analysis

The results were subjected to statistical analysis (SAS) by using two factors completely randomized design. The mean values were also tested and separated by using Duncan's Multiple Range Test (DMRT) as described by Montyonery and Douglas, (2001).
CHAPTER FOUR

4. RESULTS AND DISCUSSION

4.1 Proximate chemical composition of beef Agashi

The proximate chemical composition of beef Agashi product from different Locations (open and closed areas) in Khartoum state was carried out.

4.1.1 Moisture content

Table (1) shows the moisture contents of beef Agashi product in open and closed areas. In Omdurman they were 38.67% and 39.36%, respectively. In Khartoum they were 35.74% and 39.83%, respectively. In Khartoum North they were 35.14% and 38.74%, respectively. The values within the open and closed areas and between open and closed areas in different locations are significantly different. Sulieman et al. (2012) reported that the moisture content of cooked Agashi ranged between 32.76% and 40.00%.

4.1.2 Fat content

Table (2) shows the fat contents of beef Agashi product in open and closed areas. In Omdurman they were 5.01% and 5.76%, respectively. In Khartoum they were 5.45% and 6.09%, respectively. In Khartoum North they were 4.89% and 5.96%, respectively. These results are close to that reported by Sulieman et al. (2012) who reported that the fat contents of cooked Agashi ranged between 4.58% and 12.50%.

4.1.3 Protein content

Table (3) shows the protein contents of Agashi product in open and closed areas. In Omdurman they were 30.06% and 14.16%, respectively. In Khartoum they were 26.25% and 15.75%, respectively. In Khartoum North they were 18.04% and 22.07%, respectively. From that attained by Sulieman et al. (2012) who reported that the protein content of cooked Agashi ranged between 14.00% and 30.07%.
Table 1: Moisture content (%) of beef Agashi from open and closed Production areas

<table>
<thead>
<tr>
<th>Location</th>
<th>Open area</th>
<th>Closed area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Omdurman</td>
<td>38.67&lt;sup&gt;c&lt;/sup&gt;± 0.49</td>
<td>39.36&lt;sup&gt;b&lt;/sup&gt;± 0.51</td>
</tr>
<tr>
<td>Khartoum</td>
<td>35.74&lt;sup&gt;d&lt;/sup&gt;± 0.52</td>
<td>39.83&lt;sup&gt;a&lt;/sup&gt;± 0.64</td>
</tr>
<tr>
<td>Khartoum North</td>
<td>35.14&lt;sup&gt;c&lt;/sup&gt;± 0.26</td>
<td>38.74&lt;sup&gt;c&lt;/sup&gt;± 0.23</td>
</tr>
<tr>
<td>Lsd&lt;sub&gt;0.05&lt;/sub&gt;</td>
<td>0.2447**</td>
<td></td>
</tr>
<tr>
<td>SE±</td>
<td>0.07071</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ±SD

Mean values in columns and rows sharing the same superscript(s) are not significantly (P>0.05) different according to DMRT.
Table 2: Fat content (%) of beef Agashi from open and closed production areas

<table>
<thead>
<tr>
<th>Location</th>
<th>Open area</th>
<th>Closed area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Omdurman</td>
<td>5.01&lt;sup&gt;c&lt;/sup&gt; ± 0.47</td>
<td>5.76&lt;sup&gt;c&lt;/sup&gt; ± 0.49</td>
</tr>
<tr>
<td>Khartoum</td>
<td>5.45&lt;sup&gt;d&lt;/sup&gt; ± 0.52</td>
<td>6.09&lt;sup&gt;a&lt;/sup&gt; ± 0.55</td>
</tr>
<tr>
<td>Khartoum North</td>
<td>4.89&lt;sup&gt;f&lt;/sup&gt; ± 0.39</td>
<td>5.96&lt;sup&gt;b&lt;/sup&gt; ± 0.48</td>
</tr>
<tr>
<td>Lsd&lt;sub&gt;0.05&lt;/sub&gt;</td>
<td></td>
<td>0.07738**</td>
</tr>
<tr>
<td>SE±</td>
<td></td>
<td>0.02236</td>
</tr>
</tbody>
</table>

Values are mean ±SD

Mean values in columns and rows sharing same the superscript(s) are not significantly (P>0.05) different according to DMRT.
Table 3: Protein content (%) of beef Agashi from open and closed production areas

<table>
<thead>
<tr>
<th>Location</th>
<th>Open area</th>
<th>Closed area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Omdurman</td>
<td>30.6&lt;sup&gt;c&lt;/sup&gt; ± 0.05</td>
<td>14.16&lt;sup&gt;b&lt;/sup&gt; ± 0.22</td>
</tr>
<tr>
<td>Khartoum</td>
<td>26.25&lt;sup&gt;b&lt;/sup&gt; ± 0.50</td>
<td>15.75&lt;sup&gt;a&lt;/sup&gt; ± 0.17</td>
</tr>
<tr>
<td>Khartoum North</td>
<td>18.4&lt;sup&gt;d&lt;/sup&gt; ± 0.07</td>
<td>22.7&lt;sup&gt;c&lt;/sup&gt; ± 0.20</td>
</tr>
<tr>
<td>Lsd&lt;sub&gt;0.05&lt;/sub&gt;</td>
<td></td>
<td>0.134&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>SE±</td>
<td></td>
<td>0.03873</td>
</tr>
</tbody>
</table>

Values are mean ±SD

Mean values in columns and rows sharing the same superscript(s) are not significantly (P>0.05) different according to DMRT.
4.1.4 Ash content

Table (4) shows the ash content of beef Agashi product in open and closed areas. In Omdurman they were 3.07% and 3.77%, respectively. In Khartoum they were 2.75% and 3.79%, respectively. In Khartoum North they were 3.38% and 5.32%, respectively. The values within Omdurman and Khartoum closed area samples they are not significantly (p>0.05) different. These results are close to that reported by Sulieman et al. (2012) who reported that the Ash content of cooked Agashi ranged between 3.27% and 8.55%.

4.1.5 Fibre content

Table (5) shows the fibre content of beef Agashi product in open and closed areas. In Omdurman they were 11.39% and 10.41%, respectively. In Khartoum they were 4.39% and 5.16%, respectively. In Khartoum North they were 3.81% and 4.90%, respectively. According to Sulieman et al. (2012) who reported that the Fiber content of cooked Agashi ranged between 0.43% and 0.62%.

4.1.6 Carbohydrate content

Table (6) shows the carbohydrate content of beef Agashi product in open and closed areas. In Omdurman they were 40.05% and 48.83%, respectively. In Khartoum they were 43.29% and 40.39%, respectively. In Khartoum North they were 52.03% and 51.85%, respectively. In Khartoum North the values between open and closed area samples they are not significantly (p>0.05) different. On the other hand Sulieman et al. (2012) found that the soluble Carbohydrate content of cooked Agashi ranged between 15.5% and 43.02%.
Table 4: Ash content (%) of beef Agashi from open and closed production areas

<table>
<thead>
<tr>
<th>Location</th>
<th>Open area</th>
<th>Closed area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Omdurman</td>
<td>3.07d ± 0.19</td>
<td>3.77b ± 0.28</td>
</tr>
<tr>
<td>Khartoum</td>
<td>2.75c ± 0.16</td>
<td>3.79b ± 0.29</td>
</tr>
<tr>
<td>Khartoum North</td>
<td>3.38c ± 0.21</td>
<td>5.32a ± 0.41</td>
</tr>
<tr>
<td>Lsd$_{0.05}$</td>
<td></td>
<td>0.07738**</td>
</tr>
<tr>
<td>SE±</td>
<td></td>
<td>0.02236</td>
</tr>
</tbody>
</table>

Values are mean±SD

Mean(s) in columns and rows sharing same superscript(s) are not significantly (P>0.05) different according to DMRT.
Table 5: Fibrecontent (%) of beef Agashi from open and closed production areas

<table>
<thead>
<tr>
<th>Location</th>
<th>Open area</th>
<th>Closed area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Omdurman</td>
<td>11.39±0.56</td>
<td>10.41±0.50</td>
</tr>
<tr>
<td>Khartoum</td>
<td>4.39±0.22</td>
<td>5.16±0.41</td>
</tr>
<tr>
<td>Khartoum North</td>
<td>3.81±0.17</td>
<td>4.90±0.28</td>
</tr>
<tr>
<td>Lsd0.05</td>
<td>0.07738**</td>
<td></td>
</tr>
<tr>
<td>SE±</td>
<td>0.02236</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean±SD

Mean values in columns and rows sharing the same superscript(s) are not significantly (P>0.05) different according to DMRT.
Table 6: Carbohydrate content (%) of beef Agashi from open and closed production areas

<table>
<thead>
<tr>
<th>Location</th>
<th>Open area</th>
<th>Closed area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Omdurman</td>
<td>$40.05^{c±} 0.69$</td>
<td>$48.83^{b±} 0.70$</td>
</tr>
<tr>
<td>Khartoum</td>
<td>$43.29^{c±} 0.71$</td>
<td>$40.39^{d±} 0.65$</td>
</tr>
<tr>
<td>Khartoum North</td>
<td>$52.03^{a±} 0.85$</td>
<td>$51.85^{a±} 0.83$</td>
</tr>
<tr>
<td>$Lsd_{0.05}$</td>
<td><strong>0.2189</strong></td>
<td></td>
</tr>
<tr>
<td>SE±</td>
<td><strong>0.06325</strong></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean±SD

Mean values in columns and rows sharing the same superscript(s) are not significantly (P>0.05) different according to DMRT.
4.2 Microbial properties of beef Agashi

4.2.1 Effect of open and closed area on total viable count (cfu/g) of beef Agashi

Table (7) shows that total count is significantly (P>0.05) different in Omdurman, Khartoum and Khartoum North open and closed area. The open area samples are of higher than the closed area samples. Khartoum and Khartoum North open area samples shows no significant (P>0.05) difference. The closed area samples significantly differ in count of bacteria. On the other hand Sulieman et al. (2012) found a range of 7.5×10^3 - 6.4×10^4 (cfu/g).

4.2.2 Effect of open and closed area on yeasts and moulds counts of beef Agashi

Table (8) yeasts and moulds in Omdurman and Khartoum open area samples are not significantly (P>0.05) different. This is through the values 2.65 and 2.71 (cfu/g), respectively. On the other hand no yeasts and moulds were detected in Khartoum North open area samples and in all closed area samples. Sulieman et al. (2012) found 8.7×10^2 - 1.3×10^2 - 2.3×10^4 (cfu/g).

4.2.3 Effect of open and closed area on Staphylococcus aureus count (cfu/g) of beef Agashi

Table (9) shows higher content of Staphylococcus aureus in Omdurman, Khartoum and Khartoum North open area samples. In Khartoum and Khartoum North open area samples there was no significant (P>0.05) difference. In Omdurman closed area samples Staphylococcus aureus counted 2.54 (cfu/g). But in Khartoum and Khartoum North closed area samples counts no Staphylococcus aureus. However Sulieman et al. (2012) found 1.0×10^2 - 1.2×10^2 (cfu/g).
Table 7: Effect of open and closed area on total viable count (log$\text{}_{10}$ cfu/g) of beef Agashi

<table>
<thead>
<tr>
<th>Location</th>
<th>Open area</th>
<th>Closed area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Omdurman</td>
<td>6.73$^a$ ± 0.58</td>
<td>4.91$^c$ ± 0.73</td>
</tr>
<tr>
<td>Khartoum</td>
<td>5.80$^b$ ± 0.59</td>
<td>2.85$^e$ ± 0.41</td>
</tr>
<tr>
<td>Khartoum North</td>
<td>5.88$^b$ ± 0.59</td>
<td>3.76$^d$ ± 0.45</td>
</tr>
<tr>
<td>Lsd$_{0.05}$</td>
<td></td>
<td>0.07956**</td>
</tr>
<tr>
<td>SE±</td>
<td></td>
<td>0.02582</td>
</tr>
</tbody>
</table>

Values are mean±SD

Mean values in columns and rows sharing the same superscript(s) are not significantly (P>0.05) different according to DMRT.
Table 8: Effect of open and closed area on yeasts and moulds count (log$_{10}$ cfu/g) of beef Agashi

<table>
<thead>
<tr>
<th>Location</th>
<th>Open area</th>
<th>Closed area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Omdurman</td>
<td>2.65$^a$ ± 0.48</td>
<td>0.00$^c$ ± 0.00</td>
</tr>
<tr>
<td>Khartoum</td>
<td>2.71$^a$ ± 0.50</td>
<td>0.00$^c$ ± 0.00</td>
</tr>
<tr>
<td>Khartoum North</td>
<td>0.00$^c$ ± 0.00</td>
<td>0.00$^c$ ± 0.00</td>
</tr>
<tr>
<td>Lsd$_{0.05}$</td>
<td></td>
<td>0.1488$^{NS}$</td>
</tr>
<tr>
<td>SE±</td>
<td></td>
<td>0.0483</td>
</tr>
</tbody>
</table>

Values are mean±SD

Mean values in columns and rows sharing the same superscript(s) are not significantly (P>0.05) different according to DMRT.
Table 9: Effect of open and closed area on *Staphylococcus aureus* count (log$_{10}$ cfu/g) of beef Agashi

<table>
<thead>
<tr>
<th>Location</th>
<th>Open area</th>
<th>Closed area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Omdurman</td>
<td>3.71$^a$ ± 0.25</td>
<td>2.54$^b$ ± 0.19</td>
</tr>
<tr>
<td>Khartoum</td>
<td>2.62$^b$ ± 0.23</td>
<td>0.00$^c$ ± 0.00</td>
</tr>
<tr>
<td>Khartoum North</td>
<td>2.54$^b$ ± 0.19</td>
<td>0.00$^c$ ± 0.00</td>
</tr>
</tbody>
</table>

Lsd$_{0.05}$ 0.1488**

SE± 0.0483

Values are mean±SD

Mean values in columns and rows sharing the same superscript(s) are not significantly (P>0.05) different according to DMRT.
4.2.4 Effect of open and closed area on total coliforms count of beef Agashi

Table (10) shows the effect of open and closed area on total coliforms of beef Agashi with different significance (P>0.05). Omdurman, Khartoum and Khartoum North open area samples showed high number of total coliforms but less in Omdurman closed area samples. There was no significant (P>0.05) difference between Omdurman and Khartoum open area samples and within Khartoum and Khartoum north closed area samples. In Omdurman closed area sample coliforms counted and the value is 2.00(MPN). In Khartoum and Khartoum North closed area samples of coliforms are not detected. Sulieman et al. (2012) found 1.8×10-2.3×10² (cfu/g).

4.2.5 Effect of open and closed area on E.coli count of beef Agashi

Table (11) shows higher content of E.coli count in Omdurman, Khartoum and Khartoum North open area samples, while samples of closed areas in Omdurman, Khartoum and Khartoum North showed no E.coli counted and they are not significantly (p>0.05) different. In Omdurman and Khartoum open area samples they are no significantly (p>0.05) different. However Sulieman et al. (2012) found four positive and one negative.

4.2.6 Effect of open and closed area on Salmonella of beef Agashi

Table (12) shows the presence of Salmonella in beef Agashi. It was positive in Omdurman open area samples, while in Khartoum and Khartoum North open area samples there was no Salmonella. On the other hand Salmonella was not detected in Omdurman, Khartoum and Khartoum North closed area samples. To Sulieman et al. (2012) found three samples negative and two samples positive.
Table 10: Effect of open and closed area on total coliforms (MPN) of beef Agashi

<table>
<thead>
<tr>
<th>Location</th>
<th>Open area</th>
<th>Closed area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Omdurman</td>
<td>14.33^b ± 0.10</td>
<td>2.00^c ± 0.03</td>
</tr>
<tr>
<td>Khartoum</td>
<td>13.00^b ± 0.09</td>
<td>0.00^c ± 0.00</td>
</tr>
<tr>
<td>Khartoum North</td>
<td>21.67^a ± 0.18</td>
<td>0.00^c ± 0.00</td>
</tr>
<tr>
<td>Lsd\textsubscript{0.05}</td>
<td></td>
<td>2.685**</td>
</tr>
<tr>
<td>SE\pm</td>
<td></td>
<td>0.8714</td>
</tr>
</tbody>
</table>

Values are mean±SD

Mean values in columns and rows sharing the same superscript(s) are not significantly (P>0.05) different according to DMRT.
Table 11: Effect of open and closed area on *E. coli* (MPN/g) of beef Agashi

<table>
<thead>
<tr>
<th>Location</th>
<th>Open area</th>
<th>Closed area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Omdurman</td>
<td>$4.33^{b} \pm 0.17$</td>
<td>$0.00^{c} \pm 0.0$</td>
</tr>
<tr>
<td>Khartoum</td>
<td>$2.33^{b} \pm 0.06$</td>
<td>$0.00^{c} \pm 0.0$</td>
</tr>
<tr>
<td>Khartoum North</td>
<td>$7.33^{a} \pm 0.41$</td>
<td>$0.00^{c} \pm 0.0$</td>
</tr>
<tr>
<td>Lsd$_{0.05}$</td>
<td></td>
<td>$2.179^{*}$</td>
</tr>
<tr>
<td>SE ±</td>
<td></td>
<td>$0.7071$</td>
</tr>
</tbody>
</table>

Values are mean±SD

Mean values in columns and rows sharing the same superscript(s) do not differ significantly (P>0.05) according to DMRT.
Table 12: Effect of open and closed area on *Salmonella* of beef Agashi

<table>
<thead>
<tr>
<th>Location</th>
<th>Open area</th>
<th>Closed area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Omdurman</td>
<td>+ve</td>
<td>-ve</td>
</tr>
<tr>
<td>Khartoum</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>Khartoum North</td>
<td>-ve</td>
<td>-ve</td>
</tr>
</tbody>
</table>
4.2.7 Sensory evaluation of cooked beef Agashi

Table (14) shows the colour of cooked beef Agashi product in open and closed areas. In Khartoum and Khartoum North open areas they were not significantly (p>0.05) different, while the colour in Omdurman is different. The colour between Omdurman open and closed areas, were not significantly (p>0.05) different.

Table (14) shows the flavour of cooked beef Agashi product in open and closed areas. The flavour in Khartoum and Khartoum North open area samples was not significantly (p>0.05) while is different between Omdurman open and closed area samples.

Table (14) shows the taste of cooked beef Agashi product in open and closed areas. In Khartoum and Khartoum North open areas was not significantly (p>0.05) different, while the taste in Omdurman is different. The taste between Omdurman open and closed area samples were not significantly (p>0.05) different.

Table No. (14) Shows the general acceptability of cooked beef Agashi product in open and closed areas. The general acceptability within Omdurman, Khartoum and Khartoum North open areas, were differing significantly, while between Omdurman and Khartoum North open and closed area samples are the same.
Table No 13: Effect of open and closed area on colour, flavor, taste and general acceptability on cooked beef Agashi

<table>
<thead>
<tr>
<th>Location</th>
<th>Colour Scores</th>
<th>Flavour Scores</th>
<th>Taste Scores</th>
<th>General acceptability Scores</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Open area</td>
<td>Closed area</td>
<td>Open area</td>
<td>Closed area</td>
</tr>
<tr>
<td>Omdurman</td>
<td>3.375±0.0</td>
<td>0.1555±0.0</td>
<td>3.313±0.0</td>
<td>0.1555±0.0</td>
</tr>
<tr>
<td></td>
<td>4.813±0.0</td>
<td>0.1537±0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Khartoum</td>
<td>1.438±0.0</td>
<td>1.500±0.0</td>
<td>1.375±0.0</td>
<td>1.500±0.0</td>
</tr>
<tr>
<td>North</td>
<td>2.063±0.0</td>
<td>2.000±0.0</td>
<td>1.939±0.0</td>
<td>1.850±0.0</td>
</tr>
<tr>
<td>Lsd0.05</td>
<td>0.6277*</td>
<td>0.6277*</td>
<td>0.6784*</td>
<td>0.6204*</td>
</tr>
<tr>
<td>SE±</td>
<td>0.2199</td>
<td>0.2199</td>
<td>0.2377</td>
<td>0.2174</td>
</tr>
</tbody>
</table>

Values are mean±SD
Mean(s) in columns and rows sharing same superscript(s) are not significantly (P>0.05) different according to DMRT.
CHAPTER FIVE

5. CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

1. Agashi product showed poor microbiological quality since the microbial load in many cases exceeds the allowed levels. This could be attributed to the low quality of meat used, in its preparation and also may be due to the insufficient cooking temperature used in the process for elimination of pathogenic microorganisms such as *E. coli*.
2. The consumption of such product may lead to serious health problems.
3. The open areas (streets, transportation places) showed high number of all types of microorganisms compared with closed area.
4. The closed areas (big restaurants, modern restaurants) showed low number of microorganisms.

5.2 Recommendations

1. It is highly recommended to use high quality meat for processing into different products.
2. Agashi should be cooked properly so as to eliminate spoilage and pathogenic microorganisms, because the meat is a highly perishable food and subject to several changes.
3. The utensils used during the preparation of Agashi should be made from stainless steel in order to make cleaning easy.
4. Further study is needed to highlight the problems associated with other meat products handling, preparation, cooking and distribution and its influence on the final product quality.
5. Personal should be healthy with legal health cards to be renewed every six months.
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Appendices

Appendix 2: Moisture content (%) of beef Agashi from open and closed production areas.
Appendix 3: Fat content (\%) of beef Agashi from open and closed production areas.
Appendix 4: Crude protein (%) of beef Agashi from open and closed production areas.
Appendix 5: Ash content (%) of beef Agashi from open and closed production areas.
Appendix 6: Crude fibre (%) of beef Agashi from open and closed production areas.
Appendix 7: Carbohydrate content (%) of beef Agashi from open and closed production areas.
Appendix 8: Effect of open and closed area on total viable count ($\log_{10}$ cfu/g) of beef Agashi.
Appendix 9: Effect of open and closed area on yeasts and moulds count ($\log_{10}$ cfu/g) of beef Agashi
Appendix 10: Effect of open and closed area on *Staphylococcus aureus* count ($\log_{10} \text{cfu/g}$) of beef Agashi
Appendix 11: Effect of open and closed area on *E. coli* (MPN/g) of beef Agashi
Appendix 12: Effect of open and closed area on colour on cooked beef Agashi
Appendix 13: Effect of open and closed area on flavour on cooked beef Agashi
Appendix 14: Effect of open and closed area on taste on cooked beef Agashi
Appendix 15: Effect of open and closed area on general acceptability on cooked beef Agashi