Sudan University of Science and Technology

College of Graduate Studies

Nutritional Value and Microbiological Quality of (Agashi) Product

القيمة الغذائية والجودة الميكروبيولوجية لمنتج الأقاشي

By

Yassin Abdallah Yahia Adam

B.Sc. Agric. (honour) 2010 In Food Science and Technology

Sudan University of Science and Technology

Dissertation Submitted to Sudan University of Science and Technology in Partial Fulfillment for the Requirements of the Degree of Master of Science in Food Science and Technology

Supervisor

Prof. Dr. Ahmed Elawad Elfaki

Department of Food Science and Technology

College of Agricultural, Studies

June, 2016

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بسم الله الرحمن الرحيم

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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.

My deepest thanks and appreciations are genuinely expressed to my supervisor **Prof. Ahmed Elawad Elfaki** for his continuous

assistance, valuable advices, patience and encouragement through the course of this study.

Special thanks and sincere appreciations are due to Mr. Eltag, Mr. Ahmed Hassan and Mr. Ibrahim Ishag .

God bless all those who helped me and placed their valuable time and knowledge during the course of the study.

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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% -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 aures*, *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 لتحديد المكونات الكيميائية , القيمة الغذائية والجودة الميكروبيولوجيه لشرائح الأقاشي المستهلك بنسب كبيرة في السودان. تم أخذ العينات من ثلاثة أماكن مختلفة بولاية الخرطوم (أمدرمان, الخرطوم, الخرطوم بحري) من أماكن مفتوحة ومغلقة.

تم دراسة المكونات الكيميائية لشرائح الأقاشي ولقد اظهرت النتائج أن شرائح الأقاشي تحتوي على نسبة رطوبة (38.7%- 35.74%- 35.14% و 39.83%- 39.86%- 38.74%) 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% و 15.75%- 14.16%- 22.7%) في الأماكن المفتوحة والمغلقة في أمدرمان, الخرطوم , الخرطوم بحري، على التوالي.

نسبة الرماد (3.07% - 2.75% - 3.38% و 3.77% - 3.79% - 5.32%) في الأماكن المفتوحة والمغلقة في امدرمان, الخرطوم , الخرطوم بحري ،على التوالي.

نسبة الالياف (11.39%- 4.39% - 3.81% و 10.41% - 5.16% - 4.90%) في الأماكن المفتوحة والمغلقة في أمدرمان, الخرطوم , الخرطوم بحري ،على التوالي.

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

أيضا أظهرت نتائج التحليل الميكروبي لهذه العينات وجود مستويات عالية من العد البكتيري الكلي, بكتريا القولون, الخمائر والاعفان في الأماكن المفتوحة. وقد اظهرت هذه النتائج وجود السالمونيلا في الاماكن المفتوحة مقارنة مع الاماكن المغلقة.

هذا التلوث العالي للاقاشي المطبوخ بالميكروبات المفسدة و الممرضة أدى الى تقليل جودة المنتج وسلامة الغذاء وجعله غير ملائم للاشتراطات الصحية.

الخواص الحسية أظهرت وجود فروقات معنوية بين عينات الأماكن المفتوحة والمغلقة . عينات أمدرمان حصلت على نسبة قبول أقل من قبل المحكمين.

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, 4.8millions camel, 39.5 millions sheep and 30.8 million goats; the lates report indicate 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

2.1Meat 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).**

2.2.2Nutritional 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).**

2.2.2.1Protein

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).**

2.2.2.2Fat 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).**

2.2.2.3Minerals

Minerals are one of the classes of essential nutrients in the humandiet **(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 22release 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).**

2.2.2.4Vitamins

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) **(Romans*et 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).**

2.2.2.5Carbohydrate 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).**

2.2.2.6Pigments

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).**

2.2.2.7Enzymes

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

2.3Meat 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 valueadded meat products **(Allen *et al.*, 1998).**

2.3.1Colour

Colour of meat is an important quality attributes that influences consumer acceptance of meat and meat products **(Judge *et al.*,1990).**Consumers prefer bright-red colour for fresh meats, brown-gray color for cooked meats and pink colour for cured meats **(Jo *et al.*, 2000).**Consumers choose meat products based on the visual appearance **(Barbut, 2005).**

2.3.2Flavour

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, biter, 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).**

2.3.3Juiciness

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).**

2.3.4Tenderness

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).**

2.3.5Water 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).**

2.3.6Firmness 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).**

2.4Deterioration 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 *etal*., 1990).**

2.4.1Chemical 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 *etal*., 1990).**

2.4.2Physical 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 *etal*., 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 *etal*., 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 *etal*., 1990).**

2.5Microbial 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*etal*., 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 anerobically 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).**

2.6Microbiology 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).**

2.6.1Escherichia 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).**

2.6.2Staphylococci 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).**

2.6.3Salmonella

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 Baccilluscholerasuis, otherwise known as *Salmonella enteric***(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 **(Koohmaraie, 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 preslaughter and the environmental temperature; exercise preslaughter 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 andGrandin, 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).**

2.8.2Lipid 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 **(Fernindez*et 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 placeenzymatically or non-enzymatically. The enzymatichydrolysis of fats is termed lipolysis or fat deteriorationand is governed by specific enzymes such as lipases,estarases and phospholipase. Lipolytic enzymes couldeither be endogenous of the food product (such as milk)or derived from psychrotrophic microorganisms **(Ghaly*et al*., 2010).**

2.8.3Autolytic 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 thetenderization process **(Huss, 1995).** Post-mortemautolysis takes place in all animal tissues but atdifferent rates in different organs, quicker in glandulartissue such as the liver and slower in striated muscle**(Fearon and Foster, 1922).** The enzymes calpains,cathepsins and aminopeptidases are found to beresponsible for the post mortem autolysis of meatthrough digestion of the z- line proteins of the myofibril**(O'Halloran *et al.*, 1997).** Among theseenzymes, calpains has been described as a preliminarycontributor to the proteolytic tenderization process ofmeat. Cathepsins were, also, found to contribute totenderization at low pH. Proteolytic enzymes are active at lowtemperatures (5°C) which lead to deterioration of meatquality due to growth of microbes and biogenic aminesproduction **(Kuwahara and Osako, 2003).**

2.9Preservation 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 andnonthermal 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 *etal.*, 2004).**

2.9.1Low 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).**

2.9.1.1Chilling

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,**

1995). Chilling is critical for meat hygiene, safety, shelf life, appearance and nutritional quality **(Zhou *et al*., 2010).**

2.9.1.2Freezing

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).**

2.9.1.3Super chilling

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

2.9.2Controlled water activity methods

Microbiological safety of food is directly influenced by the water activity (a_w). The term water activity (a_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).**

2.9.2.1Sodium chloride

Borch *et al*. (1996) stated that salt-sensitive microorganisms, such as *Pseudomonas spp*. and *Eriterobacferiuceae,* did not grew in meat when the water activity (aw) was reduced from 0.99 to0.97 with the addition of 4% sodium chloride.

2.9.2.2Sugars

Chirife(1994) reported that sucrose restrained the growth of *Staphylococcus aureus* by lowering water activity.

2.10Chemical 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 *et al.*, 1997).** Traditional methods forpreservation of meat by salting and pickling are wellaccepted procedures. Other chemicals have been usedas food additives for preservation of meat but everycountry has drawn its rules and regulations and establishedlimits for the purpose of prevention of harmful effects tohuman **(Cassens, 1994).**

2.10.1Sodium 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).**

2.10.2Nitrites

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 **(Jay*et al.*, 2005).**

2.10.3Lactic acid

Lactic acid has shown antimicrobial activities against many pathogenic organisms such as *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).**

2.10.5Benzoic 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 meatpreservatives for inhibiting bacteria and fungi **(Davison *et al*., 2005).**

2.11Chemical 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).**

2.11.1Phosphates

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).**

2.11.2Salts

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

2.11.3Acids

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 *etal*., 1992).** Meat proteins represent the major functional ingredients which serve as the natural emulsifying agent in a meat emulsion **(Alvarez *etal*. 2007).** The stability of a meat emulsion is affected by both the type and amount of protein in these phases **(Gordon *etal*., 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 *etal.*, 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).**

2.13Storage 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 *etal*.,(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.

2.14Consumption of processed meats– what is the issue

The variation in processed meat products on the market is huge. In general the term processedmeat 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 productsavailable and

consumed in Denmark are produced from red meat. Red meat is in most casesand in the present work defined as meat of four legged animals. Meat from these animals has ahigher content of myoglobin and therefore also appears redder than e.g. poultry and turkeymeat 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 generalrule the EU legislation allows the addition of 150 mg nitrite per kg meat whereas the DanishNational Provisions allows the addition of 60 mg nitrite per kg meat**(Santarelli *et al.*, 2008).**

2.14.1Health 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, andwashed 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 passinganelectrical current through the brain inducing an epileptic seizue. This" head only" stun does not, however, stop the heart and so actual slaughter is achieved by severing 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 phosphrylation 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 aconceguance 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 **(Mills*eta*l., 2007).**

2.16Processed 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 *etal.,* 1990).** Many commercial products being as ground meat, chuncks 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 *etal.,* 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 *etal.*,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).**

2.17Some types of meat products

2.17.1Sausages

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).**

2.17.2Frankfurter

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).**

2.18Meat 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 **(Funge*etal*., 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 *etal.*, 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 *etal.*, 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 coli*0157.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.

2.19Agashi 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 *etal.*, 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

$$\text{Moisturecontent \%} = \frac{(M2 - M3)}{(M2 - M1)} \times 100$$

Where:

M1= weighed dish+ cover

M2= weight of dish + cover +sample before drying

M3= 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 methodsof **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) andcovered with cotton that previously extracted with hexane.Then ,the sample and a pre-dried and weighed erelenmeyer 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 dropsof 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°Cfor 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 :

$$\text{Fat content (\%)} = \frac{(W1 - W2)}{W3} \times 100$$

Where:

W1= weight of flask and ether extract

W2 = weight of empty flask

W3= 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 andconversionof 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 thee 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 catalysit (No33064,BDH,Germany) and 30 ml

concentrated sulphuric acid into kjeldahi digestion flask .After that ,the flask was placed into a 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 (Bromocreasol green and methyl red) until a brown reddish colour was observed.

Calculation

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

Where :

TV= actual volume of HCL used for sample

N= normality of HCL

F= protein conversion factor =6.25

3.2.4Ash 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,Sheffeild ,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

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

3.2.5Crude 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 m1 of H_2SO_4 (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 porclain 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, 20m1 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 contentwas 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 byHarrigan (1998). One ml of every dilution was transferred aseptically into sterile plate and to each plate10 - 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. Acolony 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 usingthe 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}. and10^{-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 Duraham tubes is recorded as positive presumptive test **(Harrigan,1998).**

3.3.5 Confirmed test for coliforms

From every tube showing positive result, atube of Brilliant Green(2%) BileBroth was inoculated by using sterile loop. The tubes were incubated at 44°C for 48hours. 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 atat 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 37^0 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°Cfor 24 hours. Then with a loop full streaking was done on solidified bismuth sulphite agar in plates were then incubated at 37°Cfor 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***(Hrrigan, 1998).**

3.4 Sensory evaluation

Sensory evaluation was done as described by **Ranganna (2001).** Using tthe 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

4.1Proximatechemical 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) showsthe 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 *etal.*(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% and5.76%, 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)whoreported that the fat contents of cooked Agashi ranged between 4.58% and 12.50%.

4.1.3Protein content

Table (3) shows the protein contents of Agashi product in open and closed areas.In Omdurman they were 30.06% and14.16%, respectively.In Khartoum they were 26.25% and15.75%, respectively.In Khartoum North they were 18.04% and 22.07%, respectively. From that attained by Sulieman *et al.* (2012) whoreported 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

Location	Open area	Closed area
Omdurman	$38.67^{c \pm} 0.49$	$39.36^{b \pm} 0.51$
Khartoum	$35.74^{d \pm} 0.52$	$39.83^{a \pm} 0.64$
Khartoum North	$35.14^{e \pm} 0.26$	$38.74^{c \pm} 0.23$
$Lsd_{0.05}$	0.2447^{**}	
SE±	0.07071	

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

Location	Open area	Closed area
Omdurman	$5.01^{e \pm}$ 0.47	$5.76^{c \pm}$ 0.49
Khartoum	$5.45^{d \pm}$ 0.52	$6.09^{a \pm}$ 0.55
Khartoum North	$4.89^{f \pm}$ 0.39	$5.96^{b \pm}$ 0.48
$Lsd_{0.05}$	0.07738^{**}	
SE±	0.02236	

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

Location	Open area	Closed area
Omdurman	$30.6^{c \pm}$ 0.05	$14.16^{b \pm}$ 0.22
Khartoum	$26.25^{b \pm}$ 0.50	$15.75^{a \pm}$ 0.17
Khartoum North	$18.4^{d \pm}$ 0.07	$22.7^{c \pm}$ 0.20
$Lsd_{0.05}$	0.134^{**}	
SE±	0.03873	

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) whoreported that the Ash content of cooked Agashi ranged between3.27% and 8.55%.

4.1.5 Fibre content

Table (5) showsthe fiber 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) whoreported that the Fiber content of cooked Agashi ranged between 0.43% and 0.62%.

4.1.6Carbohydrate content

Table (6)shows thecarbohydrate 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 fromopen and closed production areas

Location	Open area	Closed area
Omdurman	$3.07^{d} \pm 0.19$	$3.77^{b \pm} 0.28$
Khartoum	$2.75^{e} \pm 0.16$	$3.79^{b} \pm 0.29$
Khartoum North	$3.38^{c \pm} 0.21$	$5.32^{a \pm} 0.41$
$Lsd_{0.05}$	0.07738^{**}	
SE±	0.02236	

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

Location	Open area	Closed area
Omdurman	$11.39^{a} \pm 0.56$	$10.41^{b} \pm 0.50$
Khartoum	$4.39^{e} \pm 0.22$	$5.16^{c} \pm 0.41$
Khartoum North	$3.81^{f} \pm 0.17$	$4.90^{d} \pm 0.28$
$Lsd_{0.05}$	0.07738^{**}	
SE±	0.02236	

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

Location	Open area	Closed area
Omdurman	$40.05^{e \pm}$ 0.69	$48.83^{b \pm}$ 0.70
Khartoum	43.29^{c} ± 0.71	40.39^{d} ± 0.65
Khartoum North	52.03^{a} ± 0.85	51.85^{a} ± 0.83
$Lsd_{0.05}$	0.2189^{**}	
SE±	0.06325	

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.

4.2Microbial 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 *etal*. (2012) found arange of 7.5×10^{3}- 6.4×10^{4}(cfu/g).

4.2.2Effect 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 2.65and 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 *etal*. (2012) found 8.7×10^{2}- 1.3×10^{2}- 2.3×10^{4}(cfu/g).

4.2.3Effect 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). Butin Khartoum and Khartoum North closed area samples counts no *Staphylococcus aureus*. However Sulieman *etal*. (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_{10} cfu/g) of beef Agashi

Location	Open area	Closed area
Omdurman	$6.73^{a} \pm 0.58$	$4.91^{c} \pm 0.73$
Khartoum	$5.80^{b \pm} 0.59$	$2.85^{e} \pm 0.41$
Khartoum North	$5.88^{b} \pm 0.59$	$3.76^{d} \pm 0.45$
$Lsd_{0.05}$	0.07956^{**}	
SE±	0.02582	

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

Location	Open area	Closed area
Omdurman	$2.65^{a} \pm 0.48$	$0.00^{c} \pm 0.00$
Khartoum	$2.71^{a} \pm 0.50$	$0.00^{c} \pm 0.00$
Khartoum North	$0.00^{c} \pm 0.00$	$0.00^{c} \pm 0.00$
$Lsd_{0.05}$	0.1488^{NS}	
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.

Table 9: Effect of open and closed area on *Staphylococcusaureus* count (log_{10} cfu/g) of beef Agashi

Location	Open area	Closed area
Omdurman	$3.71^{a} \pm 0.25$	$2.54^{b} \pm 0.19$
Khartoum	$2.62^{b} \pm 0.23$	$0.00^{c} \pm 0.00$
Khartoum North	$2.54^{b} \pm 0.19$	$0.00^{c} \pm 0.00$
$Lsd_{0.05}$	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.

4.2.4Effect of open and closed area on total coliformscount 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 *etal*.(2012) found 1.8×10-2.3×10^3(cfu/g).

4.2.5Effect 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 Suliman*etal*. (2012) foundfour 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 waspositive 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 *etal*. (2012) found three samples negative and two samplespositive.

Table 10: Effect of open and closed area on total coliforms (MPN) of beef Agashi

Location	Open area	Closed area
Omdurman	$14.33^{b} \pm 0.10$	$2.00^{e \pm} 0.03$
Khartoum	$13.00^{b} \pm 0.09$	$0.00^{c} \pm 0.00$
Khartoum North	$21.67^{a \pm} 0.18$	$0.00^{c} \pm 0.00$
$Lsd_{0.05}$	2.685^{**}	
SE±	0.8714	

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

Location	Open area	Closed area
Omdurman	$4.33^{b} \pm 0.17$	$0.00^{c} \pm 0.0$
Khartoum	$2.33^{b} \pm 0.06$	$0.00^{c} \pm 0.0$
Khartoum North	$7.33^{a} \pm 0.41$	$0.00^{c} \pm 0.0$
$Lsd_{0.05}$	2.179^{*}	
SE±	0.7071	

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

Location	Open area	Closed area
Omdurman	+ve	-ve
Khartoum	-ve	-ve
Khartoum North	-ve	-ve

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

Location	Colour		Flavour		Taste		General acceptability	
	Open area	Closed area	Open area	Closed area	Open area	Closed area	Open area	Closed area
	Scores							
Omdurman	3.375^{a} ±0.0	0.1555^{c} ±0.0	3.313^{a} ±0.0	0.1555^{c} ±0.0	3.313^{a} ±0.0	0.1681^{c} ±0.0	4.813^{a} ±0.0	0.1537^{d} ±0.0
Khartoum	1.438^{b} ±0.0	1.500^{b} ±0.0	1.375^{b} ±0.0	1.500^{b} ±0.0	1.688^{b} ±0.0	1.750^{b} ±0.0	3.625^{bc} ±0.0	3.250^{c} ±0.0
Khartoum North	2.063^{b} ±0.0	2.000^{b} ±0.0	1.938^{b} ±0.0	1.850^{b} ±0.0	2.125^{b} ±0.0	1.875^{b} ±0.0	4.000^{b} ±0.0	4.000^{b} ±0.0
$Lsd_{0.05}$	0.6277^{*}		0.6277^{*}		0.6784^{*}		0.6204^{*}	
SE±	0.2199		0.2199		0.2377		0.2174	

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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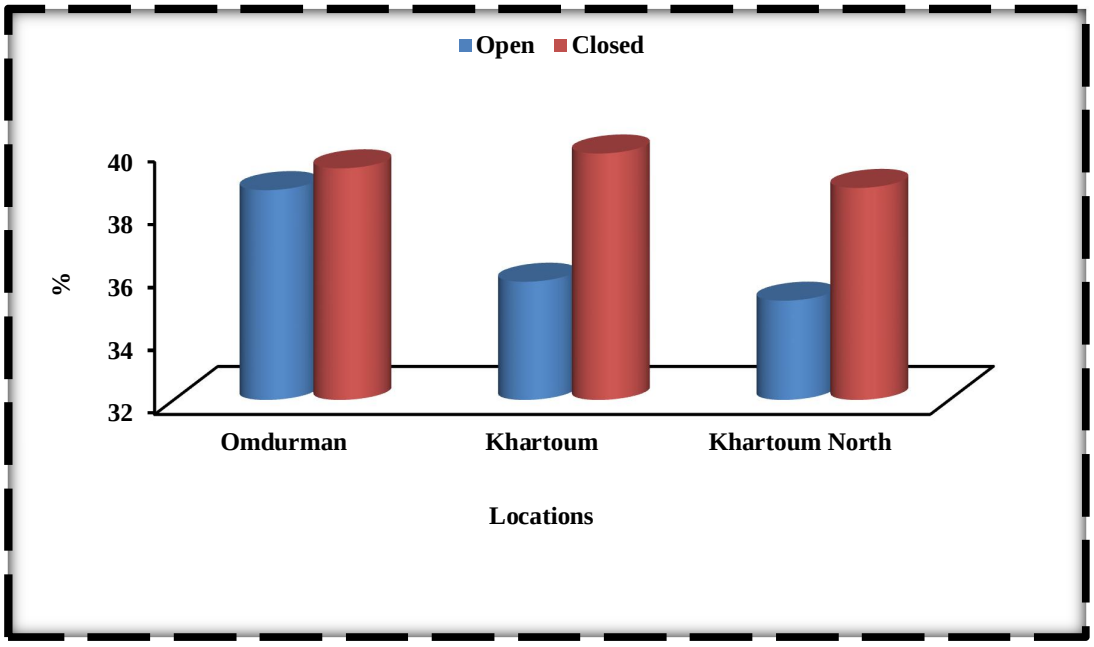
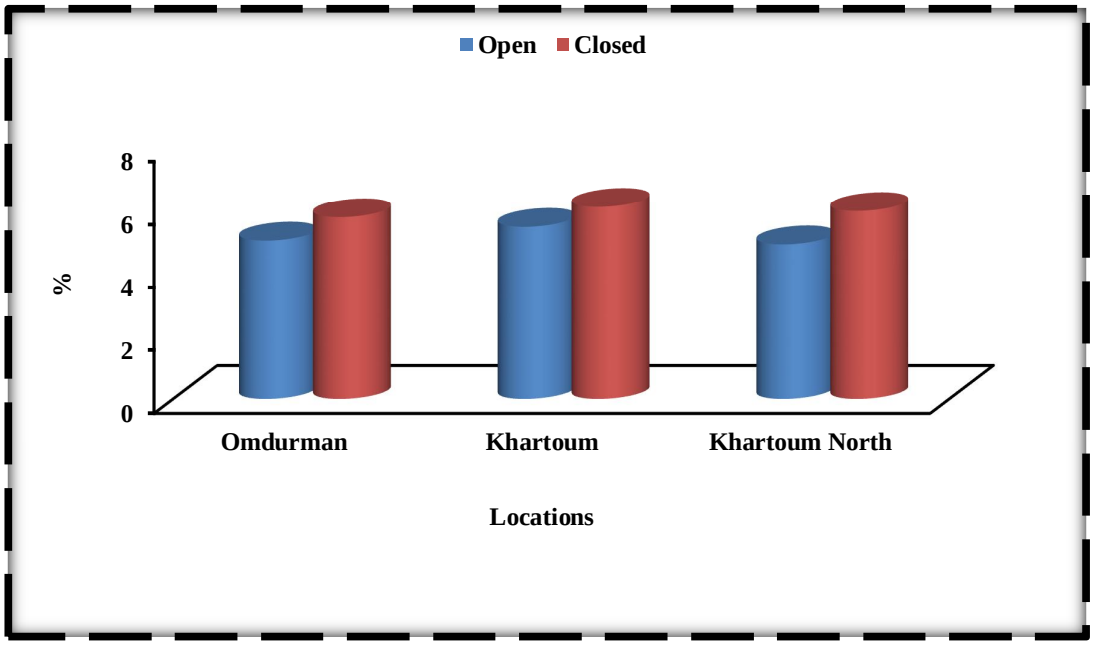
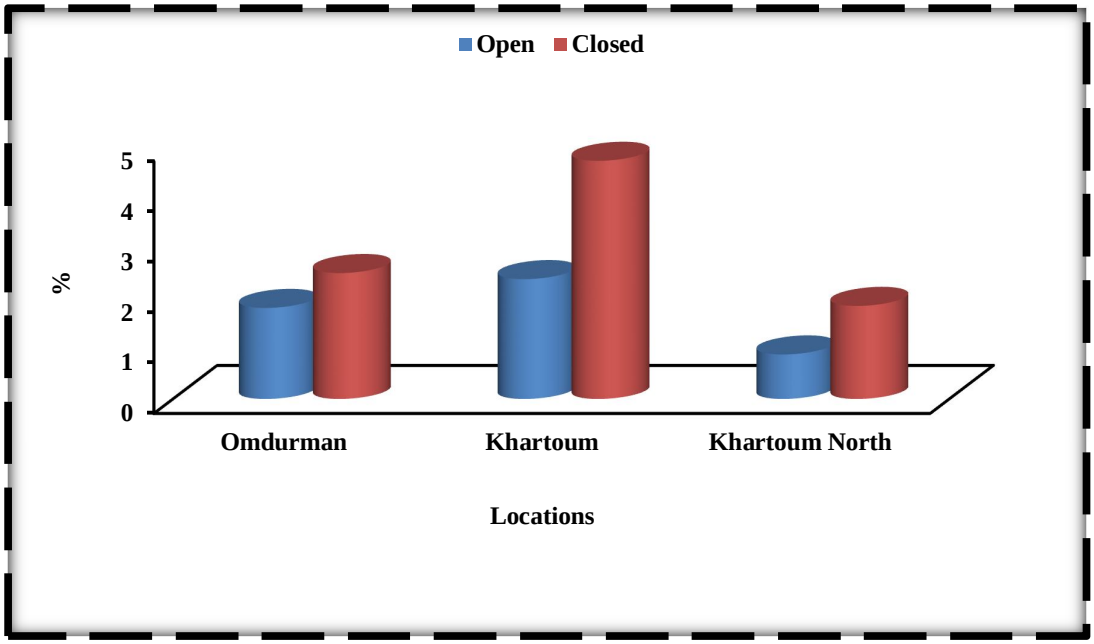
Appendices



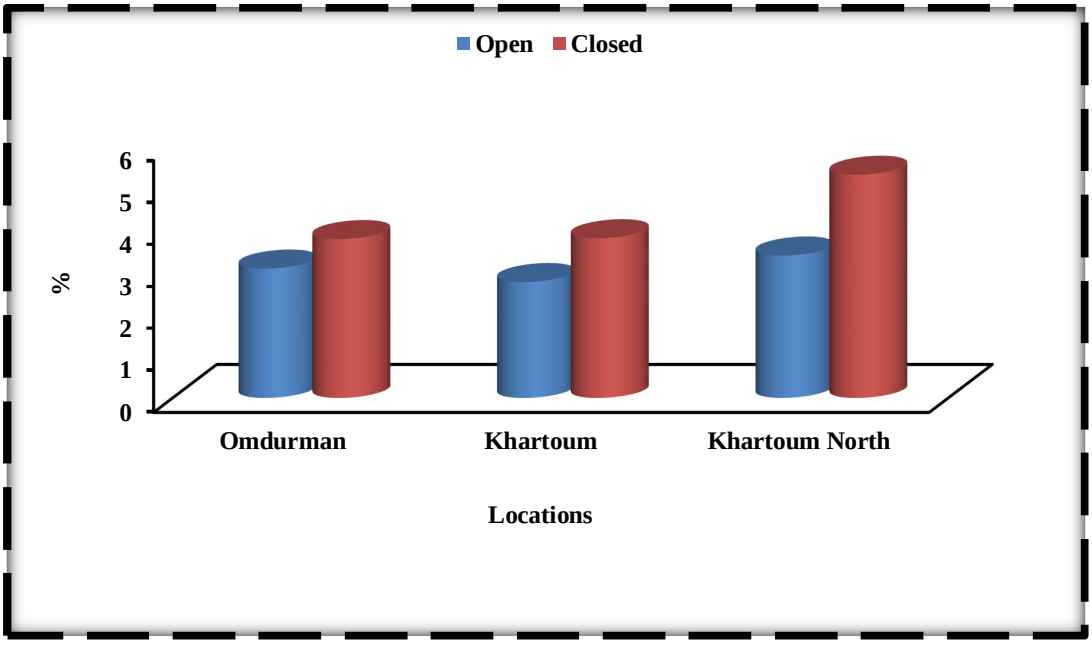
Appendix2: Moisture content (%) of beef Agashi from open and closed production areas.



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.

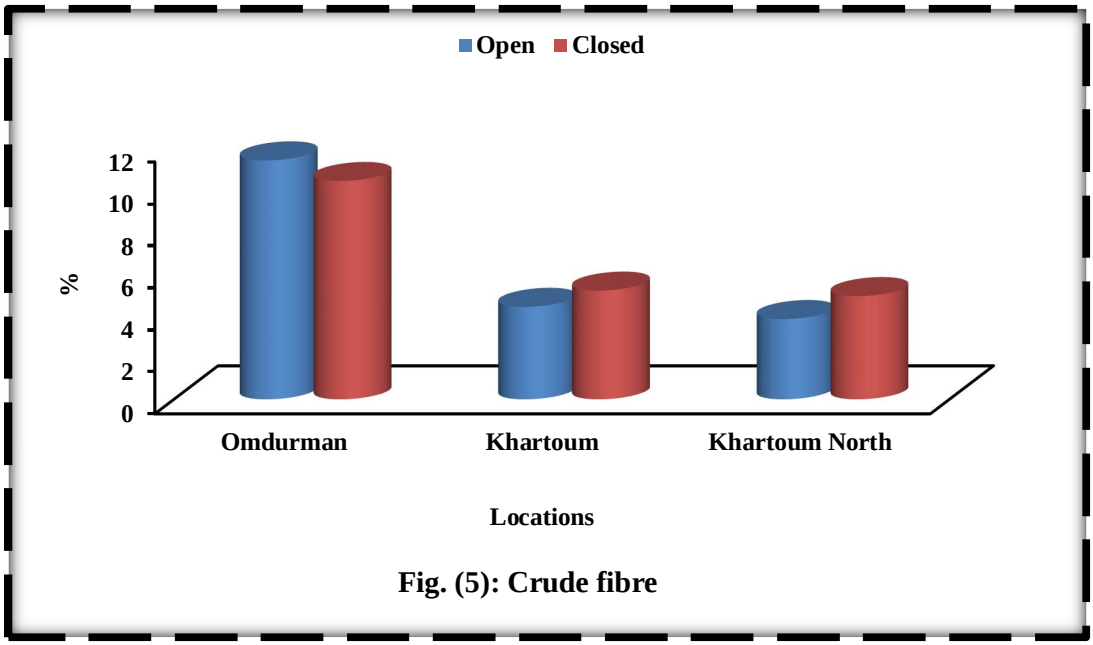
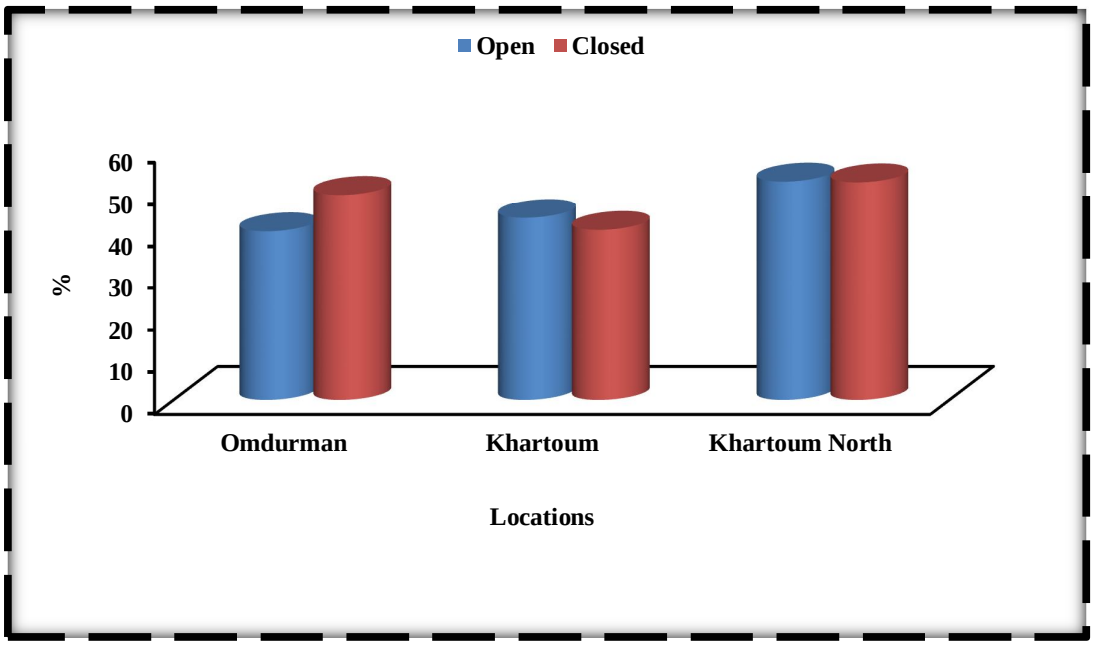


Fig. (5): Crude fibre

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.

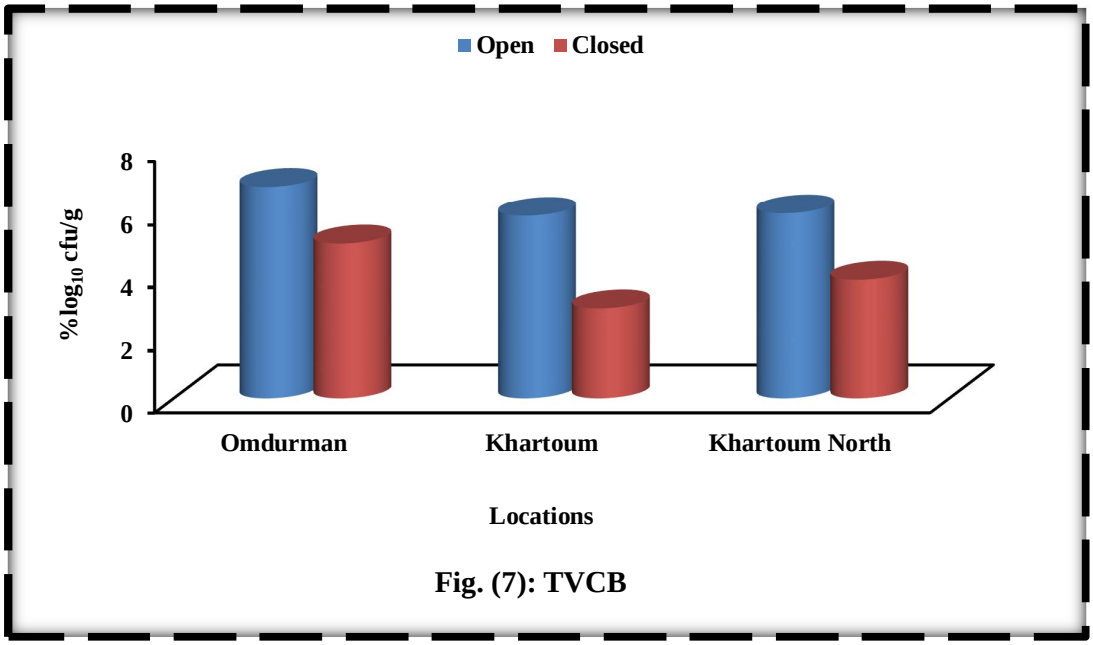
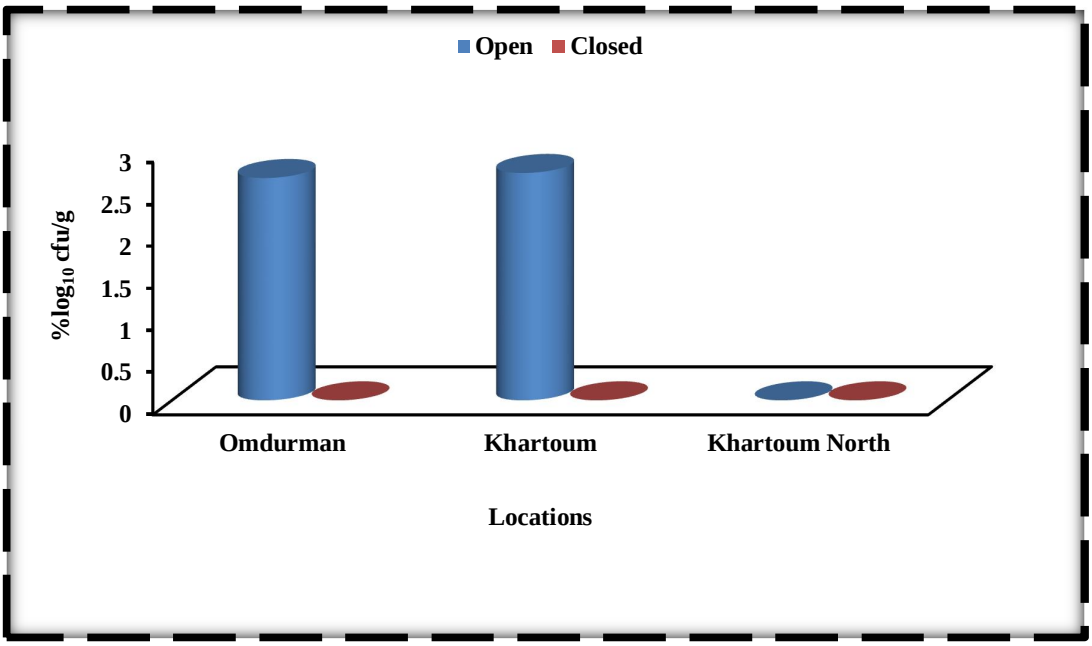
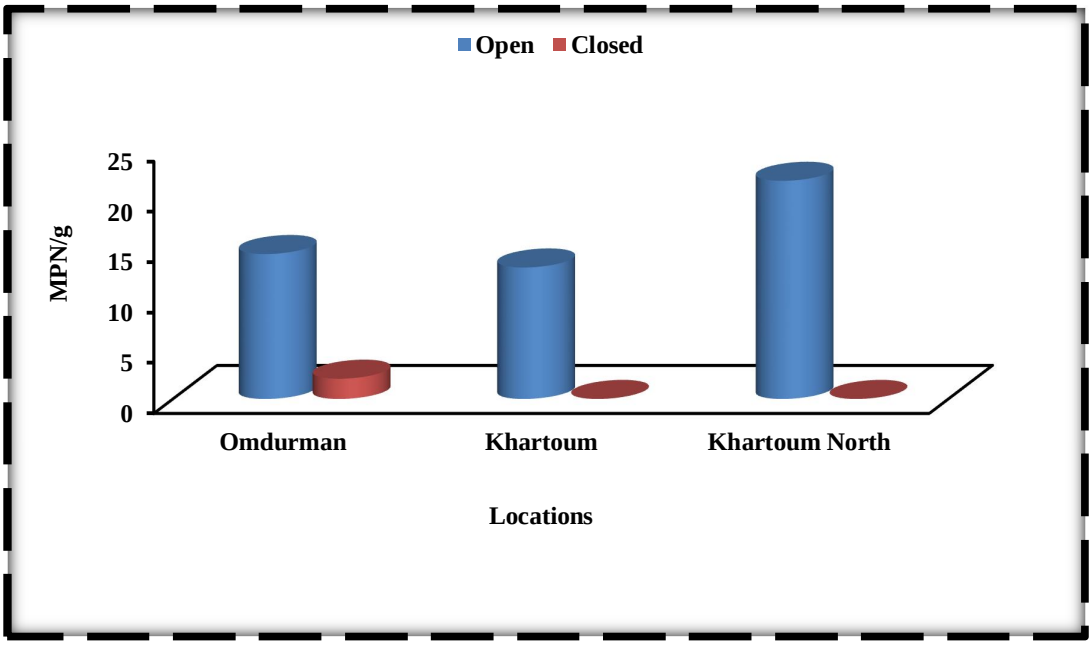


Fig. (7): TVCB

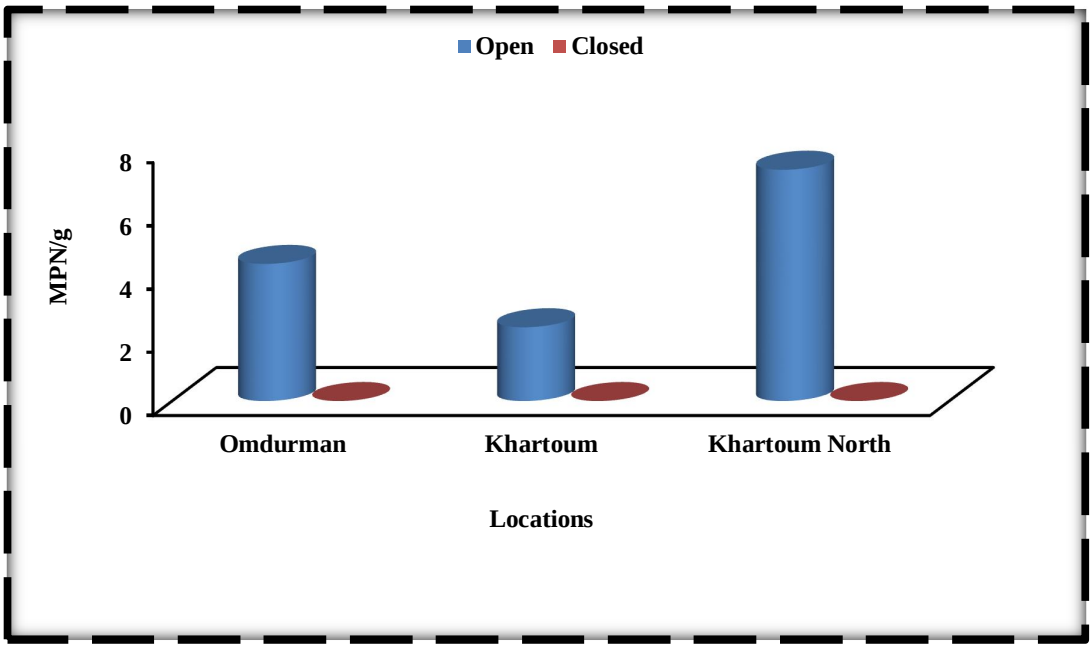
Appendix 8: Effect of open and closed area on total viable count (log_{10} cfu/g) of beef Agashi.



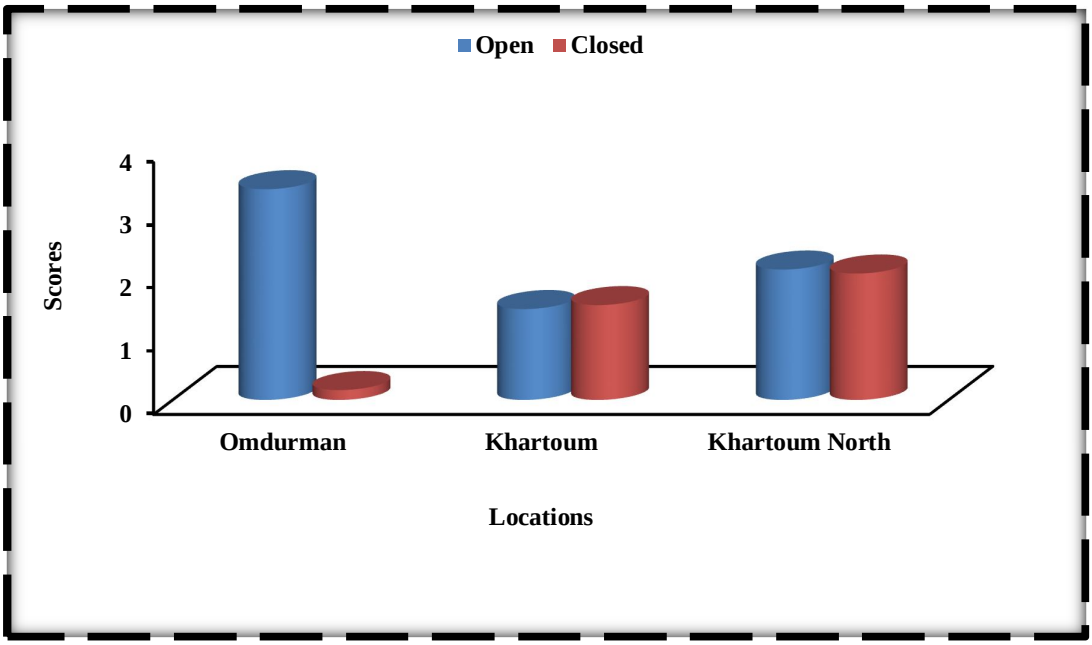
Appendix 9: Effect of open and closed area on yeasts and moulds count (log_{10} cfu/g) of beef Agashi



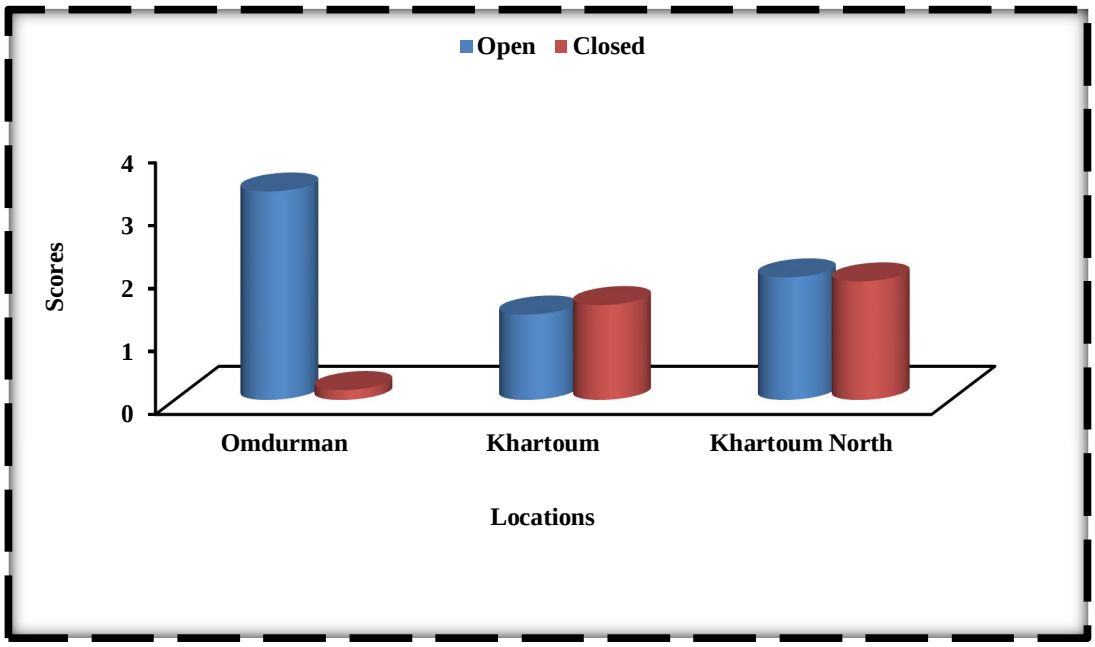
Appendice 10: Effect of open and closed area on *Staphylococcusaureus* count (log_{10} cfu/g) of beef Agashi



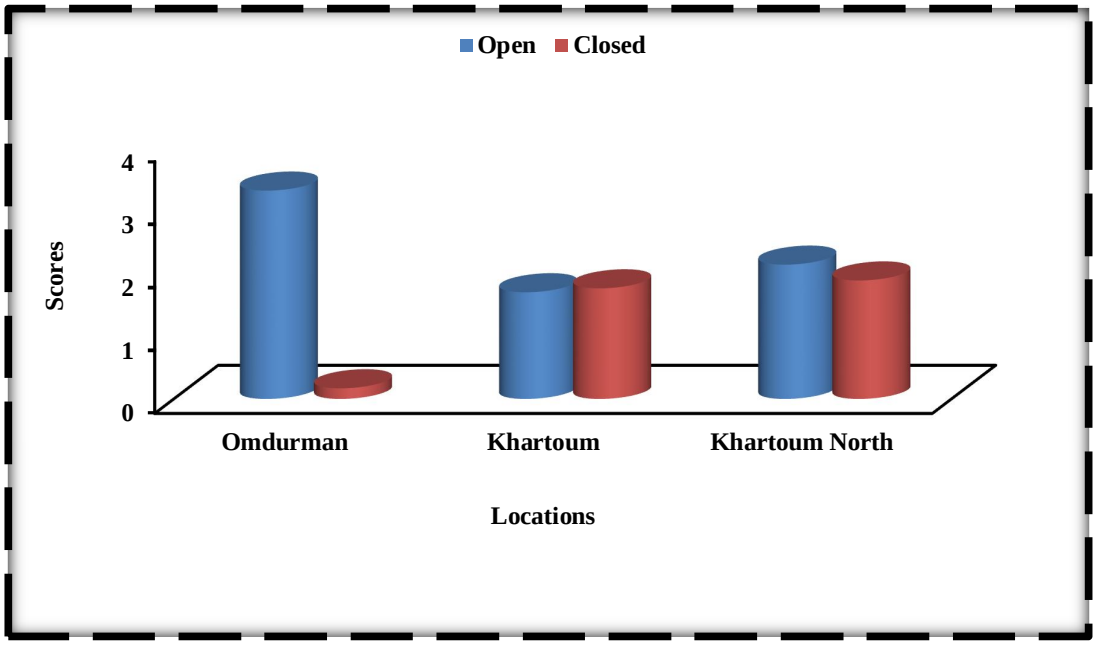
Appendix 11: Effect of open and closed area on *E. coli* (MPN/g) of beef Agashi



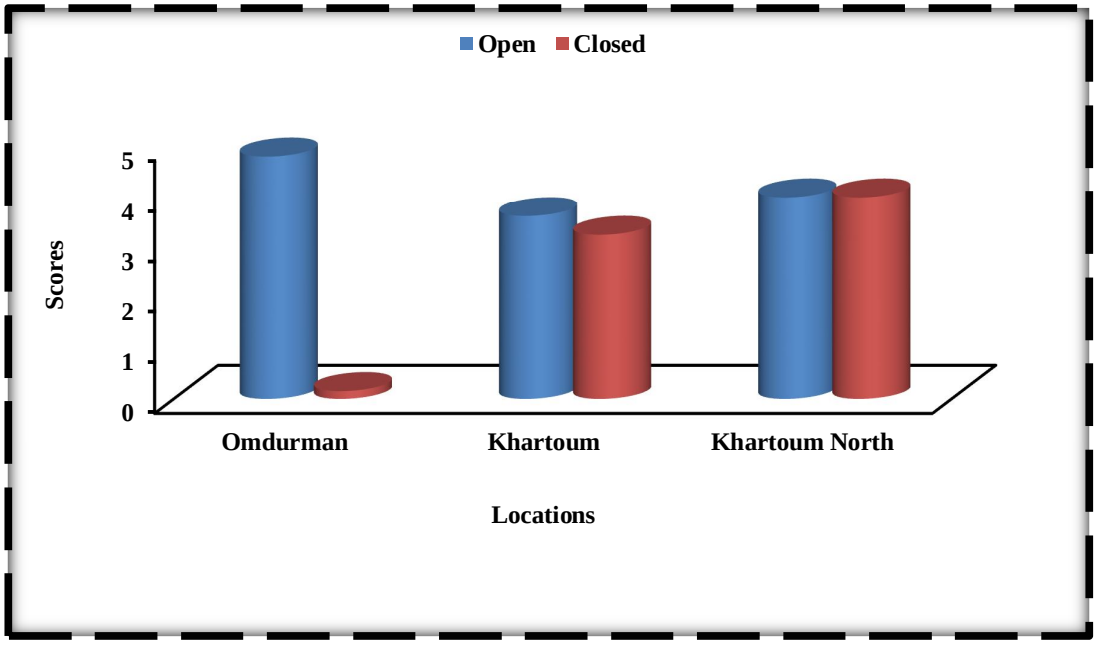
Appendix 12: Effect of open and closed area on colour on cooked beef Agashi



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