QUALITY CHARACTERISTICS AND MICROBIAL STATUS OF BEEF SMOKED WITH DIFFERENT PLANT MATERIALS AND SUYA PRODUCED FROM ROUND MUSCLES

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By

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DEPARTMENT OF ANIMAL SCIENCE,

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NIGERIA.

DECEMBER, 2014

DECLARATION

I declare that this thesis titled "**Quality Characteristics and Microbial Status of Beef Smoked with Different Plant Materials and Suya Produced from Round Muscles**" has been carried out by me in the Department of Animal Science, Ahmadu Bello University, Zaria, under the supervision of Dr (Mrs) O.O. Olusola and Prof. G.S. Bawa. The information derived from the literature has been duly acknowledged in the text and the list of references provided. No part of this thesis was previously presented for another degree or diploma at any University.

| --- | --- | --- |
| Bala Reuben GANDI | **Signature** | **Date** |

CERTIFICATION

This thesis entitled "**Quality Characteristics and Microbial Status of Beef Smoked with Different Plant Materials and Suya Produced from Round Muscles**" by **Reuben Bala GANDI** meets the regulations governing the award of the degree of Master of Science in Animal Science of Ahmadu Bello University, Zaria, Nigeria and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This thesis is dedicated to the Sovereign Lord-who is my always present help and also to my dear Family.

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ABSTRACT

Two experiments were conducted. The first evaluated beef smoked using different plant materials (*Acacia raddiana, Eucalyptus camaldutensis, Azadirachta indica* and *Cocos nucifera*) as source of smoke. The effect of the plant materials on the organoleptic, microbial and physicochemical properties of smoked beef was evaluated. In the second study, Suya was produced from various round muscles (*Rectus femoris, Semi-tendinosus, Biceps-femoris, Semi membranosus* and *Vastus lateralis*) and evaluated for organoleptic, microbial and physicochemical properties. Both studies were carried out in a completely randomized design. The result showed that there was no significant (P>0.05) difference among sources of fuel wood tested on the overall acceptability of smoked meat. However, organoleptic scores on a five point hedonic scale were lowest (2.50) for beef smoked with C. *nucifera* and highest (3.30) for beef smoked with *A. raddiana* (Standard check). The pH values were within the accepted limit (5.5-6.5). Percentage thermal shortening were not significantly (P>0.05) different. Percentage Water holding capacity was highest (13.6) in beef smoked with *C. nucifera*. Total viable counts/ Aerobic plate count, coli-form counts were all within satisfactory limits (i.e. <½ million/g). In the second study, the fat content was not significantly (P>0.05) affected by the muscle types. The score for overall acceptability indicated that the consumers preferred *Biceps femoris* which was significantly (P< 0.05) different from other round muscles. Water holding capacity was observed to have influence on other qualities such as the flavour, juiciness and tenderness. Product yield was lowest (70.20 %) in *Vastus lateralis*, indicating a good yield from all muscles. Microbial load of all suya samples fell within satisfactory limit with reference to the standard microbial load specification (i.e. <½ million/g). It was concluded that *Eucalyptus camaldutensis* (Turare), *Azadirachta indica* (Neem) and *Cocos nucifera* (coconut shell) are good sources of fuel wood and can be used as an alternative to *Acacia raddiana* for smoking beef. Furthermore, smoke had an antimicrobial and antioxidant effects as the microbial load were all within satisfactory limit. From the results of the second study, it was concluded that the prime cuts, apart from resulting in Suya with high prices are not necessarily better than Suya from less choice parts of the carcass (*Semi-membranous, Semi-tendinosus, Biceps femoris, Rectus femoris* and *Vastus lateralis*) in terms of product yield and eating qualities

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CHAPTER ONE

1.0 INTRODUCTION

Meat has been defined as the flesh of animals which is suitable as food. Meat makes a valuable contribution to diets because of its high biological value and an excellent source of amino acids, vitamins and minerals (CAST, 1997). A daily intake of 100 g of meat can supply up to 50% of the recommended daily allowance for Iron, Zinc, Selenium, Vitamins B_1, B_2, B_6, B_{12} and 100% of vitamin A (Biesalski and Nohr, 2009).

In Nigeria there is a preferential consumption of different types of meat by communities due to a combination of factors bordering on religious belief, culture, food habits, sex of animal, age at slaughter, socio-economic factors and individual variation (Ajiboye *et al.*, 2011).

Meat being nutritious with high moisture content and nearly neutral pH is a good culture medium for many micro-organisms (bacteria, yeasts and moulds) and as such, classified among perishable foods whose contamination with spoilage organisms are almost unavoidable (Ikeme, 1990). This makes meat preservation more difficult than other types of food as it may result in oxidative rancidity, discolouration, off flavour, sliminess etc. The kind and amount of spoilage organisms in meat depends upon the availability of nutrients, presence of oxygen, temperature, pH at storage and generation interval of the spoilage microorganism under given environment etc. (Forrest *et al*., 2001). It is necessary to minimize deterioration in order to prolong the time during which acceptable levels of quality are maintained. This depends upon the processing and preservative method used and the inherent properties of the meat in question (Forrest *et al*., 2001).

Different methods of meat preservation have been used through out history. These include, the use of high temperature (e.g. roasting), Low temperature (e.g. chilling, freezing and

pasteurization), Drying (e.g. hot air drying, wind and sun drying) and the use of Chemical preservatives.

Meat smoking as a method of red meat preservation dates back to prehistoric time. Smoking involves the use of wood fuel which in turn affects product quality. Smoking improves flavor and appearance of meat (Cardinal *et al.*, 2006), making them a delicacy in many communities. Smoking is normally used in combination with salting. The moisture content drops to 10-40 % depending on the smoking process. Preservation by smoking is achieved by dehydration and antibacterial effect of the smoke. During the smoking process, smoke components penetrate the meat to produce a stable and sensory acceptable product. Smoked meat has good shelf life unless rehydrated. Handling and storage methods are primarily concerned with minimizing microbial contamination and retarding microbial growth and activity (Benjakul and Aroonrueng, 1999).

The second phase of this study involves Suya, a spicy barbecued, smoked or roasted meat product. Suya is one of the Intermediate Moisture Meat (IMM) products that is easy to prepare and highly relished (Omojola *et al.,* 2004). It originated from the Hausa people of northern Nigeria, where rearing of cattle is an important occupation and a major source of livelihood for the people with the preparation process carried out under largely unhygienic conditions with high risk of contamination (Abdullahi *et al*., 2004). The humid tropical environment encourages the breeding of flies which swarm and perch on meat displayed for sale. These constitute a major nuisance and can aid in the spread of disease agents of significant public health importance (Okoli *et al.* 2006). The fact that there are sporadic cases of gastroenteritis and symptoms of food infection after consumption of Suya indicate that the product indeed constitutes a food safety risk (Odusote and Akinyanju, 2003). Similarly, most of the bacteria causing diarrhoea are zoonotic (transmissible between animals and humans) and reports by FAO/WHO (2003) shows that Cholera, Salmonellosis, Campylobacteriosis, Shigellosis,

Typhoid, Brucellosis, Poliomyelitis, and *Escherichia coli* infections are prevalent. Although cooking temperature kills most pathogen, a few may withstand the cooking temperatures. Moreso, suya can be re-contaminated by food handlers during processing or from the environment (Wagner, 2010).

1.1 Justification of the Study

In Nigeria, exact statistics of storage losses of smoked meat is not available, but observations reveal that post processing losses of smoked meat do occur during storage. Therefore, any reduction in processing and post processing loss by simple modifications of existing methods will benefit the meat sellers as well as introduce newer varieties of meat products. If found to be effective on meat, the use of *E. camaldutensis* (Turare), Neem and coconut shells as wood source may ease pressure on local trees like *A. raddiana* (Doka) and Mangroves.

Traditionally, most Suya producers use expensive cuts of meat (examples is the Longissimus dorsi muscle) resulting into high prices of the products beyond the reach of the common man. The prime cuts apart from resulting in product with high prices might not be better than cuts from less choice parts of the carcass in terms of product yield and eating qualities. Hence, the need to convert low priced meat cuts into relished processed products by meat processors.

Accurate information on the prevalence and impact of foodborne disease are lacking. Thus, there is a need to determine the occurrence and load of micro-organism from smoked beef and suya.

1.2 Objectives of the Study

1. To determine the effect of using different plant materials (*Acacia raddiana* (Doka), *Eucalyptus camaldutensis* (Turare), *Azadirachta indica* (Neem) and *Cocos nucifera* (coconut shell)) as smoke source on sensory and physical characteristics of smoked beef.
2. To determine the organoleptic properties and shelf life of beef suya made from different cut of carcass.
3. To determine the microbial and physical parameters of smoked beef and Suya.

1.3 Hypothesis

This research therefore, was designed to evaluate the following null hypothesis (H_O) and alternative hypothesis (H_a)

- Ho: Smoking of beef with different plant materials has no effect on the Organoleptic and Preservative properties of smoked beef.
- Ha: Smoking of beef with different plant materials has effect on the organoleptic and preservative properties of smoked beef.

- Ho: Suya produced from different beef cut (round muscles) have no effect on the organoleptic and Preservative properties of Suya.
- Ha: Suya produced from different beef cut (round muscles) have effect on the organoleptic and preservation properties of suya.

CHAPTER TWO

2.0 LITERATURE REVIEW

Meat is animal flesh that is eaten as food. The advent of civilization allowed the domestication of animals such as chickens, sheep, pigs and cattle, and eventually their use in meat production on an industrial scale (Womack, 2010). The meat industry is concerned with turning an animal carcass into many different end-products.These end-products are derived from all parts of the animal (muscle, bone, fat, cartilage, skin, fluids and glands) and are produced through a range of physical, chemical and biological processes.

2.1 Domestication of animals for meat

Paleontological evidence suggests that meat constituted a substantial proportion of the diet of even the earliest humans. The domestication of animals dates back to 10,000 BC (Lawrie and Ledward, 2006), allowing the systematic production of meat and the breeding of animals with a view to improving meat production. The breeding of beef cattle optimized for meat production as opposed to animal best suited for draught or dairy purposes began in the mid 18th century (Mark and William, 2001).

2.2 Growth and Development of Meat Animals

Agricultural science has identified several factors bearing on the growth and development of meat in animals. These factors include;

2.2.1 Genetic factors

Several economically important traits in meat animals are heritable to some degree and can thus be selected for by breeding. In cattle, certain growth features are controlled by recessive genes, one of such trait is dwarfism, another is the doppelender or ‘double muscling’ condition, which causes muscle hypertrophy and thereby increases the animal's commercial value.

Genetic analysis continues to reveal the genetic mechanisms that control numerous aspects of the endocrine system and through it, meat growth and quality (Alan, 2006).

Genetic engineering techniques can shorten breeding programmes significantly because they allow for the identification and isolation of genes coding for desired traits, and for the re-incorporation of these genes into the animal genome (Lawrie, 2003). To enable such manipulation, research aimed at mapping the entire genome of sheep, cattle and pigs is still on going. Lawrie (2003) reported a developed recombinant bacterium which improves the digestion of grass in the rumen of cattle and also genetically alter some specific features of muscle fibres.

2.2:2 Environmental Factors

Heat regulation in livestock is of great economic significance, because mammals attempt to maintain a constant optimal body temperature. Low temperatures tend to prolong animal development and high temperatures tend to retard it (Lawrie, 2003). Depending on their size, body shape and insulation through tissue and fur, some animals have a relatively narrow zone of temperature tolerance and others (e.g. cattle) a broad one.

2.2.3 Nutrition

The quality and quantity of usable meat depends on the animal's plane of nutrition. Scientists disagree about how exactly the plane of nutrition influences carcass composition (Schurgers and Vermeer, 2000). High quality protein feed is expensive. Hence, several techniques are employed to ensure maximum utilization of protein (Forrest *et al*, 2001). Environmental factors influence the availability of crucial nutrients or micronutrients a lack or excess of which can cause great ailments (Waxman *et al.*, 2007). In Australia for instance, where the soil contains limited phosphate, cattle are being fed additional phosphate to increase the efficiency of beef production (Ronald and Fereidoon, 2004). Plant toxins are also a risk to grazing

animals for instance, sodium fluoracetate, found in some African and ralian plants, kills by disrupting the cellular metabolism (Waxman *et al*., 2007) and some pesticide residues present a particular hazard due to their tendency to bio-accumulate in meat, potentially poisoning consumers.

2.2:4 Human Interventions

Animal producers may seek to improve the fertility of female animals through the administration of gonadotrophic or ovulation-inducing hormones. Artificial insemination is now routinely used to produce animals of the best possible genetic quality and the efficiency of this method is improved through the administration of hormones that synchronize the ovulation cycles within groups of females. Growth hormones particularly anabolic agents such as steroids, are used in some countries to accelerate muscle growth in animals. This practice has given rise to the beef hormone controversy, an international trade dispute. It may also decrease the tenderness of meat and have other effects on the composition of the muscle flesh. Where castration is used to improve control over male animals, its side effects are also counteracted by the administration of hormones (Amanda, 2013).

2.3 Meat Composition

Meat is composed of: Water, protein, fat and other water-soluble organic material (Table 2.1).

Table 2.1 Nutritive Composition of Beef

Substance	Composition	Proportion %
Water	Hydrogen, oxygen	73
Protein	Amino acids	22
Fat	Phospholipids, Cholesterol and Fat Soluble Vitamins	3.9
Other Soluble Organics	Vitamins,Carbohydrates	1.1

Source: Rendle and Keeley, (1998).

Muscle proteins are either soluble in water (sarcoplasmic proteins, about 11.5 % of total muscle mass) or in concentrated salt solutions (myofibrillar proteins, about 5.5 % of mass). There are several hundred sarcoplasmic proteins that are involved in the glycolytic pathway in the conversion of stored energy into muscle power.The two most abundant myofibrillar proteins; myosin and actin are responsible for the muscle's overall structure. The remaining protein mass consists of connective tissue (collagen and elastin) as well as organelle tissue (Moon, 2006).

Fat in meat can be either adipose tissue used by the animal to store energy and consisting of true fats (esters of glycerol with fatty acids), or intramuscular fat which contains considerable quantities of phospholipids and of unsaponifiable constituents such as cholesterol.The fat portion includes some fat-soluble substances, including some vitamins. Meat is an important source of amino acids (the building blocks of proteins), minerals, vitamins and energy (Moon, 2006).

2.4 Biochemical Changes: From Muscle to Meat

The most significant change occurring after slaughtering an animal is that blood circulation stops, the resulting effect is that oxygen is no longer sent to the animal cells. This means that reactions begin to take place under anaerobic conditions. One of the major consequences of this is that the pH decreases because in the absence of oxygen, glucose is converted to lactic acid rather than CO_2 and H_2O. Changes in key properties of the meat are outlined below (Pearson, 2012).

In life: Glycogen + $O_2 \rightarrow CO_2 + H_2O$ + ATP (energy)

But in death: Glycogen $\frac{Anaerobic}{condition}$ $Lacticacid +$ ATP

2.5 Acidity in Meat

pH is a measure of acidity or alkalinity. In beef, pH can be 5.4 to 7.0. The pH level of meat can affect the shelf life of the meat, its colour, its tenderness, and the eating quality. With the accumulation of lactic acid the pH falls from 7 to 5.5, and at the same time the energy rich ATP reserve is depleted. The drop in pH is a desirable feature as a low pH slows down growth of micro-organisms and enhances flavour, juiciness and colour of the meat to give an attractive saleable product (Pearson, 2012). The more glycogen in the muscles, the more lactic acid formed post slaughter. Meat from stressed animals hava a high pH, causing it to be Dark in colour, Firm in texture and Dry in taste (known as DFD meat).

The eating quality of meat is a function of the animal production and carcass processing that has been applied. While live animal factors (animal age, nutrition, breed, pre-slaughter stress) often have a marked effect on beef eating quality, on many cases the treatment of the carcass on the slaughter floor and in the chiller can have a far greater effect.

The combination of a very rapid fall in pH and slow cooling of the carcass can lead to heat or rigor shortening, whereas a slow fall in pH and rapid cooling can lead to cold shortening

(Pearson, 2012). Heat shortening is undesirable in terms of eating quality as it causes dryness and slight to moderate toughness. Cold shortening is also undesirable as it can cause moderate to severe toughness. A less obvious but important penalty of both cold and heat shortening is a reduction in the ability of the meat to age. Thus, the aim is to achieve an optimal rate of pH fall and/or the mechanical prevention of shortening.

Recent studies have confirmed that, if the pH drops very rapidly while the muscle temperature is high, there may be considerable fluid loss from the muscle. This in turn may lead to lack of juiciness and therefore inferior eating quality (Pearson, 2012). The same combination may also contribute to the development of two toned meat, a condition in which there are undesirable gradations in meat colour within a cut, with the deep meat tissue being paler than the normal red meat closer to the surface.

As a guide, according to Pearson (2012), if the pH is at or below 6.0 while the temperature is at, or above 35°C, then there may be heat shortening. If at any time the pH is at or above 6.0 when the temperature is at or below 12°C, then it is likely that there may be cold shortening.

2.6 Meat Quality Parameters

2.6:1 Tenderness

Tenderness is one of or the most discussed features in meat. It is a real challenge for the scientific community and for the meat industry to achieve products with standardized and guaranteed tenderness, since these characteristics are exactly what consumers want in a meat product (Koohmaraie, 1995). It is recognized that processes leading to meat tenderization are mainly enzymatic in nature and involve proteolytic systems. There are two concepts based on enzymatic biochemistry used in explaining meat tenderness; Firstly, some researchers postulated that meat tenderization is affected mainly by calpain (a calcium-activated proteases consisting of at least three proteases; μ-calpain, m-calpain and skeletal muscle-specific calpain)

which is responsible for myofibril protein degradation (Koohmaraie and Geesink, 2006). Secondly, others suggested a hypothesis that tenderization is a multi-enzymatic process corresponding to apoptosis, where calpain is an important enzyme (Koohmaraie et al., 2002). Variation exists among muscles and differences are mainly due to the amount of connective tissue in the various cuts and amount of connective tissue present is due to the function of the muscle.

2.6.2 Meat Colour

The first impression consumers have of any meat product is its color and thus color is of utmost importance. The color of meat may vary from the deep purplish-red of freshly cut beef to the light gray of faded cured pork (Rockland and Beuchat, 1997). Fortunately, the color of meat can be controlled if the many factors that influence it are understood. Fresh and cured meat color both depend on myoglobin, they are considerably different from each other in terms of how they are formed and their overall stability. Myoglobin is a water-soluble protein that stores oxygen for aerobic metabolism in the muscle. It consists of a protein portion and a nonprotein porphyrin ring with a central iron atom. The iron atom is an important player in meat color.

The defining factors of meat color are the oxidation (chemical) state of the iron and which compounds (oxygen, water or nitric oxide) are attached to the iron portion of the molecule (Kropf *et al.*, 2004). Because muscles differ greatly in activity, their oxygen demand varies. Consequently different myoglobin concentrations are found in the various muscles of the animal. Also, as the animal gets older there is more myoglobin. A greater myoglobin concentration yields a more intense color. Muscle pigment concentration also differs among animal species. For example, beef has considerably more myoglobin than pork or lamb, thus giving it a more intense color.

Immediately after cutting meat color is quite dark, beef would be a deep purplish-red. As oxygen from the air comes into contact with the exposed meat surfaces it is absorbed and binds to the iron. The surface of the meat blooms as myoglobin is oxygenated. This pigment, called oxymyoglobin, gives beef its bright cherry red color. It is the color consumers' associate with freshness (Kropf *et al.*, 2004).

2.6:3 Cooked Meat Pigment

During the cooking process, myoglobin is denatured. All of the pigment is not affected at the same time or to the same extent and this is why you get reddish color at different end point temperatures when heat is applied. The cooked pigment is denatured metmyoglobin, it is brown and is easily recognized in cooked meat products. Certain meat conditions can result in protection of the myoglobin in meat (Hunt *et.al.*, 1999).

The ultimate pH of meat or meat products will affect how the meat color changes during smoking. If the meat has a high pH, it will be cooked to higher end-point temperatures to get the same visual degree of doneness as one with normal pH. Frequently, complains of this hard to cook defect are associated with a high pH of the meat or meat product (Torngren, 2003).

2.6:4 Effect of Meat pH

The rate and extent that muscle pH declines postmortem are both variable and have a great impact on the color of meat and meat products. The normal pH decline in muscles is from approximately 7.0-7.2 down to near pH 5.5-5.7 over about 24 hrs (Sorheim *et al.*, 1999). With this pH decline, whole tissue is the characteristic color of the species. If the pH declines to the normal pH of 5.5-5.7 within 45 min or less, the muscle will appear very pale and soft (PSE). A very low ultimate pH (<5.4) will also result in a paler color (Sorheim *et al.*, 1999). If the pH does not drop much at post mortem, the meat will be dark with a dull, dry surface (DFD). As the ultimate pH increases, the meat gradually becomes darker. This darkening of color

becomes noticeable when the muscle pH exceeds 5.7.Summary of meat quality parameters are shown in the table below;

Table 2.2 Parameters of Meat Quality

Parameters	Acceptable	Unacceptable
Apperance		
Meat colour	Red/pink	Brown, Grey green
Fat colour	White	Yellow
Texture	Firm	Soft, mushy, dry
Palatability		
Tenderness	Tender	Mushy, tough
Flavor	Typical of specie	Boar taint, rancid, acid taste
Juiciness	Moist	Lack of flavor

Source: Laird (2006)

2.7 Meat Smoking

2.7.1 Composition of Smoke

Hardwoods are made up of cellulose, hemicellulose and lignin. Cellulose and hemicellulose are the basic structural material of the wood cells while lignin acts as a kind of cell-bonding glue. Some softwoods especially pines and firs hold significant quantities of resin which produces harsh-tasting soot when burned, these woods are not often used for smoking (Hui *et al.*, 2001). Cellulose and hemicellulose are aggregate sugar molecules. When burnt, they effectively caramelize producing carbonyls which provide most of the color components, sweet, flowery and fruity aromas. Lignin, a highly complex arrangement of interlocked phenolic molecules, also produce a number of distinctive aromatic elements when burnt, including smoky, spicy, and pungent compounds such as guaiacol, phenol,syringol, and sweeter scents.Guaiacol is the phenolic compound mostly responsible for the smokey taste, while Syringol is the primary

contributor to smokey aroma (Hui *et al.*, 2001). Wood also contains small quantities of proteins, which contribute to roasted flavors. Many of the odor compounds in wood smoke especially the phenolic compounds, are unstable, dissipating after a few weeks or months.

A number of wood smoke compounds act as preservatives. Phenol and other phenolic compounds in wood smoke are both antioxidants (which slows rancidity of animal fats) and antimicrobial (which slow bacterial growth). Other antimicrobials in wood smoke include Formaldehyde, Acetic Acid and other organic acids which give wood smoke a low pH of 2.5, some of these compounds are toxic to people as well, and may have health effects if found in high quantities (Kjallstrand and Petersson, 2001).

Different species of trees have different ratios of components hence, impart different flavor to food. Another important factor is the temperature at which the wood burns. High temperature fire causes the flavor molecules to break down further into unpleasant or flavorless compounds (Fabech and Larsen, 1992). Woods that contain high lignin tend to burn hot, to keep them smoldering requires restricted oxygen supplies or a high moisture content (Arnim and Yetti, 2012). When smoking using wood chips or chunks, the combustion temperature is often raised by soaking the pieces in water before placing them on a fire.

The chemical aspects of meat smoking have been reviewed (Toth and Potthast, 1984) and the chemical composition of smoke condensation has been assessed in many studies, including some investigations by Guillen and Ibargoitia (1998 and 1999, respectively) where Methoxyphenols were identified as major components and of great importance for the smoke flavour and for the preserving antioxidant effect of the smoke.

2.7.2 Plant Materials Used For Smoking

In Nigeria, reports on wood quality of various species of trees used as fuel energy for smoking meat are limited. Alien tree species that has encroached the North West region of Nigeria is *Eucalyptus Camaldutensis* (Turare), and has good potentials as a wood fuel though it has not been reported as a source of smoke for meat smoking in Nigeria. It also has edible component and can ease pressure on *A. raddiana* and *I. Doka* which are the current tree of choice for meat smoking in this area **(**Oduor *et al.*, 2008).

Coconut *(Cocos nucifera*) plantations provide numerous products such as coconut meat, milk and shells. Coconut shells can be used for cooking, heating and as the heating base for smoking meat and fish. The use of coconut shells for fish smoking was tried by Benjakul and Aroonrueng (1999), results from this study showed that fish smoked using coconut shell had good sensory qualities.

The main interest in using Neem (*Azadirachta indica*) in meat smoking is because of activity of its components as a deterrent for both insect activity and mould. It has multiple pesticidal and medicinal properties. Smoke from its' leaves are used as insect repellant. About 135 different compounds are found in every part of the tree and it also has antimicrobial effects (Battacharyya *et al*., 2007). Neem's various part reportedly have antihelmintic, antiseptic, antisyphilitic, astringent, demulcent, diuretic, emmenagogic, emollient and purgative actions and have also been used to treat boils, eye diseases, exzema, headaches, hepatitis, leprosy, rheumatism, scrofula, and ulcers (Ketkar, 1986). No study has been reported for the use of Neem tree for meat smoking in Nigeria and for control of insect infestation during storage of smoked meat.

Table 2.3 Concentrations (mg m-3) of selected components of smoke from *E. Camaldutensis* (Turare), *A.raddiana*, *A. indica* (Neem) and *C. nucifera* (coconut shell).

Smoke Components	T1	T2	T3	T4
Phenols				
2,6-D2,6-Dimethoxyphenols	16.8	16.1	24.1	14.3
2-Methoxyphenols	5.3	5.3	2.6	39
Phenol		0.9	0.2	1.7
Anhydrosugars				
1, 6-Anhydroglucose		6.0		
Form'aldehydes	0.4			
2-Furaldehyde	2.0	2.5	2.1	
5-Methyl-2-furaldehyde	0.3	0.3	0.2	
Furans				
2-Methylfuran		1.6	1.5	1.3
2,5-Dimethylfuran	0.3			
Bensofuran	0.4	0.4	0.4	
Hydrocarbons				
Benzene	2.2	2.2	1.9	3.8
Methylbenzene	1.5	1.4	3.2	1.9
Styrene	1.3	0.2	0.2	1.0

Source: Kjailstrand and Petersons (200l).

T1= Smoke from Acacia, T2= Smoke from Eucalyptus

T3= Smoke from Neem, T4= Smoke from coconut shell

2.7.3 Smoke House

The upright drum smoker (also referred to as an Ugly Drum Smoker or UDS) is exactly what its name suggests. An upright steel drum modified for the purpose of hot smoking. There are many ways to accomplish this, but the basics include the use of a complete steel drum, a basket to hold charcoal near the bottom and cooking rack near the top, all covered by a vented lid of some sort. They have been built using many different sizes of steel drums, but the most popular size is the common 55 gallon drum (Wikipedia, 2013a). The temperatures used for smoking are controlled by limiting the amount of air intake at the bottom of the drum and allowing a similar amount of exhaust out of vents in the lid. UDSs are very efficient with fuel consumption and flexible in their abilities to produce proper smoking conditions.

Smoking chambers with freshly generated smoke, according to the investigated procedure, offers several advantages. The hardwood produces smoke with a high proportion of 2,6-dimethoxyphenols, which have stronger protective antioxidant activity than 2- Methoxyphenols from softwood (KjaIlstrand and Petersson, 2001). The smoldering bed of wood particles in the smoke generator helps in keeping a constantly low combustion temperature, this results in a high proportion of the particularly anti-oxidative 2,6-methoxyphenols with an alkenyl side-chain in the smoke while it also keeps the concentrations of hazardous polycyclic aromatic compounds at a very low level. A drawback is the high concentration of benzene in the curing atmosphere, but benzene appears to be much less absorbed than the major methoxyphenols which are condensed on smoke particles (Kjällstrand and Petersson, 2010). The regulated internal temperatures for smoke cook beef is 160^0F to ensure less risk of benzene and other carcinogenic component of the smoke (USDA-FSIS, 1999).

2.7.4 Effect of Smoking on Meat

Smoking is the process of flavoring, cooking or preserving food by exposing it to the smoke from burning or smoldering plant materials most often wood. Meats and fish are the most commononly smoked foods (McGee, 2004).

Hot smoking exposes the foods to smoke and heat in a controlled environment. Like cold smoking, the item is hung first to develop a pellicle and then smoked. Although foods that have been hot smoked are often reheated or cooked, they are typically safe to eat without further cooking. Hot smoking occurs within the range of 52 to 80 °C (Myrvold, 2011). Within this temperature range, meat is fully cooked, moist and flavorful. If the smoker is allowed to get hotter than 85 °C, the meat will shrink excessively. Smoking at high temperatures also reduces yield, as both moisture and fat are cooked away. It can equally cause a toughening of meat fibres due to heat coagulation and shrinkage of the myofibrillar proteins and connective tissues. Initial toughening is due to protein denaturation which occurs when the meat reaches 50-80^{o}C, this is followed by some tenderization which occurs as collagen hydrolyses to gelatin at temperatures greater than 75^{o}C (Inneke, 2011). However, prolonged heating can increase the tenderness due to the conversion of collagen to gelatine by heating.

Preservation technique used on beef is vital as some of the nutrients can be damaged. Bangladesh (2009) compared different processing methods in relation to nutrient quality and observed the quality decreasing trend was lower in smoked cooked, flat size meat sample. The author observed that rural smoking method of meat drying could be a useful technique of meat preservation.

2.7.5 Health Risks of Smoked Meat

Evidence suggests that smoked foods may contain carcinogens (Fritz and Soos, 1991). The smoking process contaminates food with Polycyclic Aromatic Hydrocarbons (PAHs) and nitrosamines, which are known carcinogens. So, in theory consuming smoked food increases the risk of gastrointestinal cancer. Some studies have found a positive statistical correlation between intestinal tract cancer and the frequent intake of smoked foods (Fritz and Soos, 1991). The United States National Cancer Institute emphasizes that population studies have not established a definitive link between cooked meats and cancer in humans, but suggests individuals reduce their exposure to PAHs.

2.8 Suya

Due to the chemical composition and biological characteristics of meat, they are highly perishable which provide excellent environment for growth of many hazardous microorganisms that can cause infection in humans and spoilage of meat and economic loss. Hence, the need for effective, cheap and simple preservative techniques. One of such simple preservative technique is intermediate moisture food processing. Obanu *et al.* (1981), observed that Intermediate Moisture Meat, are shelf stable under the tropical climate without refrigeration and may be eaten directly with or without rehydration. Suya is one of such intermediate moisture product that is easy to prepare and highly relished (Omojola *et al.*, 2004).

Suya is a traditional meat product that is commonly produced by the Hausas in West Africa from mostly Beef. There are three types of Suya namely, Tsire, Kilishi and Balangu. Of the three types, Tsire, which is boneless meat pieces that are staked on slender wooden sticks and cooked by roasting, using a glowing fire from charcoal, is the most popular with consumers in Nigeria (Igene and Mohammed, 1993). In big cities and small towns, Suya vendors have

become very prominent with their grill stands becoming very busy from about midday until late at night (Inyang *et al*., 2005).

Meat processing generally enables the processor to convert low priced meat cuts into high priced processed products (FAO, 1995). Traditionally, most tsire suya producers use expensive cuts of meat (for examples the Longissimus dorsi muscle), resulting into high prices of the products beyond the reach of the common Man.The prime cuts, apart from resulting in products with high prices might not be necessarily better than cuts from less choice parts of the carcass in terms of product yield and eating qualities.

2.8.1 Characterization of Connective Tissue of Bovine Skeletal Muscles

Commercially important skeletal muscles of bovine carcass are grouped as (a) neck muscles, (b) shoulder muscles, (c) rib cage muscles, (d) loin muscles, (e) hind limb muscles, (f) sirloin muscles and (g) flank muscles (Swatland, 1994). The muscles of the hind limb which is of importance to this study include; *Gracilis, Sartorius, Quadriceps Femoris, Vastus medialis, Vastus lateralis, Vastus intermedius, Rectus femoris, Semi-tendinosus, Semi-membranosus, Adductor, Pectineus, Biceps femoris* and *Gastrocnemius.*

Different methods of carcass fabrication are reported, including the Conventional Approach, the Innovative cut technique (Pfeiffer *et al*., 2005) and Hot boning (Seideman and Cross, 1995). Conventional carcass fabrication includes separation of fore and hindquarters followed by separation of rib, chuck and brisket (and so on) by saw cutting. Meats from the cuts are then separated after trimming off fat. Briefly, the Innovative cut fabrication process includes in sequence, separation of fore and hindquarters of carcasses, trimming off all major connective tissues and fat and finally separation of individual muscles of each quarter from their points of attachment (Pfeiffer *et al*., 2005). In hot boning, lean meat and fat are removed from the bones prior to chilling (Seideman and Cross, 1995).

2.8:2 Cuts of Beef

Cuts of beef are first divided into primal cuts, pieces of meat initially separated from the carcass during butchering. These are basic sections from which steaks and other subdivisions are cut. Since the animal's legs and neck muscles do the most work they are the toughest. The meat becomes tenderer as distance from hoof and horn increases. Different countries and cuisine have different cuts and names, and sometimes use the same name for a different cut e.g., the cut described as 'Brisket' in the US is from a significantly different part of the carcass than British 'Brisket' (Wikipedia, 2013b).

The following is a list of the American primal cuts and cuts derived from them; Chuck, Rib, Brisket, Foreshank or Shank and plate (all found in the forequarter). The hindquarter cuts include; the Loin, Round and Flank. The Round contains lean and is moderately tough having lower fat (less marbling) cuts, which require moist or rare cooking. Some representative cuts of the round are; Top round (*Semimembranosus*), Bottom round (*Biceps femoris)*, Eye of round (*Semitendinosus*) and Sirloin tip (*Rectus Femoris*).

2.9 Meat Spoilage

The spoilage of meat occurs if the meat is untreated in a matter of hours or days and results in the meat becoming unappetizing, poisonous or infectious (Lawrie, 1990). Spoilage is caused by the practically unavoidable infection and subsequent decomposition of meat by bacteria and fungi, which are borne by the animal itself, by the people handling the meat and by their implements. Meat can be kept edible for a much longer time though not indefinitely if proper hygiene is observed during production and processing and if appropriate food preservation and food storage procedures are applied (Lawrie and Ledward, 2006)

The organisms spoiling meat may infect the animal either while still alive (endogenous disease) or may contaminate the meat after its slaughter (exogenous disease).There are numerous diseases that humans may contract from endogenously infected meat, such as

anthrax, bovine tuberculosis, brucellosis, salmonellosis, listeriosis, trichinosis or taeniasis (Ledward and lawrie, 2006).

Infected meat however, should be eliminated through systematic meat inspection. Consumers will more often encounter meat exogenously spoiled by bacteria or fungi after the death of the animal. The large intestine of animals contains some 3.3×10^{13} viable bacteria, which may infect the flesh after death if the carcass is improperly dressed (Troller and Christian, 1990) Contamination can also occur at the slaughterhouse through the use of improperly cleaned slaughter or dressing implements. After slaughter, care must be taken not to infect the meat through contact with any of the various sources of infection in the abattoir, notably the hides and soil adhering to the carcass, water used for washing and cleaning, the dressing implements and the slaughter house personnels.

Bacteria genera commonly infecting meat while it is being cut, processed, packaged, transported, sold and handled include; Salmonella spp., Shigella spp., E.*coli*, B.*proteus*, Streptococci etc. These bacteria are all commonly carried by humans, infectious bacteria from the soil include; Cl. *Botulinum*. Among the molds commonly infecting meat are *Penicillium* spp., Mucor, Cladosporium, Alterneria, Sporotrichium and Thaminidium etc. (Lawrie and Ledward, 2006)

As these microorganisms colonize a piece of meat they begin to break it down leaving behind toxins that can cause enteritis or food poisoning, potentially lethal in the rare case of botulism (Rajasekhara *et al.*, 2000). The microorganisms do not survive a thorough cooking of the meat, but several of their toxins and microbial spores do. Meat spoilage by micro-organisms can manifest itself as follows:

Table 2.4 Symptoms of Microrganism found in meat

Oxygen	Microbial agent	Symptoms
Present	Aerobic bacteria	• Surface slime • Discolouration • Gas production • Change in odor • Fat decomposition
Present	Yeasts	• Surface slime • Discoloration • Change in odor and taste • Fat decomposition
Present	Molds	• Sticky and "whiskery" surface • Discoloration • Change in odor • Fat decomposition
Absent	Anaerobic bacteria	• Putrefaction and foul odors • Gas production • Souring

Source: Lawrie (2003).

2.9.1 Contaminants of Meat

2.9.1.1 Bacteria Contaminants of Meat

During slaughtering process, there is contamination of the sterile tissue with intestinal flora such as gram-negative organisms which includes *Escherichia coli* as well as contaminants such as *Pseudomonas* specie and gram-positive lactic acid bacteria and *Staphylococci* species associated with humans, animals and their environment. Meat spoilage is usually associated with gram-negative proteolitic bacteria which literally decompose the protein with production of offensive odour (Haman, 1977).

The addition of salt and drying of fresh meat have been an effective means to control the meat microflora and thus preserve the tissue for later consumption. The curing salt (sodium chloride or sodium nitrate or sodium nitrite) and subsequent proper handling method, favours the growth of gram-positive bacteria, primarily *Staphylococcus aureus* while inhibiting the

proliferation of gram-negative bacteria (Boles *et al.,* 2000). Ogunbanwo *et al*., (2004) reported that smoking has a preservative effect on the Suya meat

As part of the normal flora of the human intestinal tract, *E. coli* plays a crucial role in food digestion by producing vitamin K from undigested material in the large intestine. Most strains of *E coli* are harmless commensal members of the intestinal flora of mammals in which some strains adhere to the intestinal mucosa while others are only temporary transient in the lumen of the colon. While some strains of *E. coli* live as commensals, many are opportunistic pathogens of humans and other animals (Levine, 1994). *E. coli* is the major cause of Neonatal septicemia, Neonatal meningititis and urinary tract infections in humans and of a variety of invasive diseases in mammals. It is also a leading cause of diarrhoeal diseases in humans and other mammals (Neidhardt, 1995). *E. coli* can respond to environmental factors such as Chemicals, pH, temperature and osmolarity in a number of ways, since it is a single celled organism (Todar, 2002).

Salmonella is a genus of rod-shaped, Gram-negative, non-spore forming predominantly motile enterobacteria with diameters around 0.8 to 1.5µm, lengths from 2 to 5µm and peritrichous flaglla, (flagella that are all around the cell body). There are only two specie of salmonella; *Salmonella bongori* and *enterica.* Salmonella is found worldwide in both cold blood and in the environment. They cause illnesses such as typhoid fever, paratyphoid fever, and food poisoning (Ryan and Ray, 2004).

2.9.1.2 Yeasts and moulds

Fungi are slow growers in comparison to bacteria and so are often out-competed seldom responsible for the spoilage of fresh proteinaceous material.Yeasts and moulds are more resistant to low temperature, low pH, lower a_w values and the presence of preservatives than bacteria (Taniwaki *et al.*, 2001).

Generally speaking however, it appears that fungal metabolism is best suited to substrates high in carbohydrates, whereas bacteria are more likely to spoil proteinaceous foods with the exception of Lactobacilli. If meat surface dries out, it will inhibit bacteria and permits mould growth. Chill foods of low pH inhibit bacteria but may allow mould/yeasts to develop (Taniwaki *et al.*, 2001).

2.9:2 Effect of Temperature on meat spoilage

The influence of temperature in food preservation and spoilage has two separate facets: Temperatures during processing and Temperature during storage (Beuchat and Toledo, 1994). As noted above heat-resistant fungal spores may survive pasteurising processes. Apart from a few important species, little information exists on the heat resistance of fungi. Low pH and preservatives increase the effect of heat (Beuchat, 1981) and also hinder resuscitation of damaged cells (Beuchat and Jones, 1995).

2.9.3 Effect of Water Activity on meat spoilage

Water availability in foods is most readily measured as water activity. Water activity (a_w) is a physicochemical concept, introduced to microbiologists by Scott (1977), who showed that a_w effectively quantified the relationship between moisture in foods and the ability of microorganisms to grow on them.

Water activity is defined as a ratio: a_w ¼ $p=p^o$

Where p= is the partial pressure of water vapour in the test material and

P^o= is the saturation vapour pressure of pure water under the same conditions.

Water activity is numerically equal to equilibrium relative humidity (ERH) expressed as a decimal (Duckworth and Ormerod, 1997). In many practical situations, a_w is the dominant environmental factor governing food stability or spoilage. Knowledge of fungal water relations will then enable prediction both of the shelf life of foods and of potential spoilage

fungi. For example; Ascomycetous fungi and conidial fungi of ascomycetous origin comprise most of the organisms capable of growth below 0.9 a_w (Taniwaki *et al.*, 2001).

2.9.4 Effect of gas tension on meat spoilage

Food spoilage moulds like filamentous fungi, have an absolute requirement for oxygen. The concentration of oxygen dissolved in a substrate has a much greater influence on fungal growth than atmospheric oxygen tension (Miller and Golding, 1989). For example, *Penicillium expansum* grows virtually in 2.1% oxygen over its entire temperature range and many other common food spoilage fungi are inhibited only slightly when grown in nitrogen atmospheres containing approximately 1.0% oxygen (Taniwaki *et al.*, 2001).Most food spoilage moulds appear to be sensitive to high levels of carbon dioxide, although there are notable exceptions. When maintained in an atmosphere of 80% carbon dioxide and 4.2% oxygen, *Penicillium roqueforti* still grew at 30% of the rate in air (Miller and Golding, 1989), provided that the temperature was above 28^0C. In 40% CO_2 and 1% oxygen, *Penicillium roqueforti* grew at almost 90% of the rate in air (Taniwaki *et al*., 2001). Xeromyces bisporus has been reported to grow in similar levels of carbon dioxide (Dallyn and Everton, 1969).

At least some species of Mucor, Rhizopus and Fusarium are able to grow and ferment in bottled liquid products and sometimes cause fermentative spoilage. Growth under these conditions may be yeast-like.

It is evident from the above discussion that the growth of fungi in a particular food is governed largely by a series of physical and chemical parameters and definition of these can assist greatly in assessing the food's stability. The situation in practice is made more complex by the fact that such factors frequently do not act independently, but synergistically. If two or more of the factors outlined above act simultaneously, the food may be safer than expected. This has been described by Leistner and Ro'del (1996) as the 'Hurdle Concept'. This concept

has been evaluated carefully for some commodities such as fermented sausages and is now widely exploited in the production of shelf stable bakery goods and acid sauces.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Location

The study was conducted at the Animal product Laboratory, Department of Animal Science, Ahmadu Bello University, Zaria. Located within the Northern Guinea Savannah Zone on the latitude 11^0 $9^{'}45^{''}$ N and Longitude $7^0$38'8''E, at altitude of 610m above sea level (0vimaps, 2012).

3.2 Sample Collection and preparation for Smoked Beef

Nine kilogram of beef-round from a freshly slaughtered 3 year old bull was obtained from the Animal Product Laboratory and used for this study. After excising muscle from carcass, meat was trimmed of all extra muscular fat and cleaned thoroughly with water. The round was held overnight for 24 hours at 4^0C, after which it was cut and sliced into pieces weighing 70 – 90 grams and 6 - 8 cm wide. The sliced pieces were sprinkled with tincture of salt.

3.3 Smoking of Meat

3.3.1 Construction of Smoking Ovens

An upright drum smoke house with stoke holes was constructed, allowing opening at one end with the top opened (vent) and closed at intervals.



3.3.2 Meat Distribution in smoke ovens

Meat smoking trays were oiled with food vegetable oil prior to meat distribution to avoid sticking. A total of 1000 gram sliced beef round were placed on trays in the smoke oven, same was done for each treatment. The trays with beef were transferred under a shade at an angle to let the meat drip dry for 20-30 minutes prior to smoking.

3.3.3 Smoke Cooking Procedures

Logs of wood from *Eucalyptus camaldutensis* (Turare), Acacia raddiana and *Azadirachta indica* (Neem) obtained from Savanna Forestry Research Institute, were cut using hand saws from chunks to give smaller pieces of 50 cm length and a fairly uniform thickness of 4.0 to 7.0 cm. The coconut shells (*C.nicifera*) were utilized the way they are when dried. The fire was lit 15 minutes before smoking started using dried grass to start the fire and allowed to burn till the flames die off and only smouldering wood remaining. The beef samples were transferred to the smoking chambers already prepared. Each set of trays with 1000 g of meat were smoked using the four tree types in each of the four ovens.

The treatments were: Meat smoked with Acacia (MA), Meat sm oked with Eucalyptus (ME), Meat smoked with Neem (MN) and Meat smoked with Coconut shell (MC).

The fire from the plant materials was controlled by closing the stoke holes during smoking to allow less air. To control the excessive temperature in the ovens, the intensity of the fire was reduced by intermittent withdrawal of some of the logs from the fire point, the temperature of the oven was measured at intervals to ensure it was within the range of 50-80^{o}C. The positions of the meat were interchanged at intervals to ensure uniform penetration. At the end of the 60-90 minutes of smoking, meat samples were removed from the kilns and exposed to air to cool for 5-10 min in accordance with procedure of Akande *et al.*, (1996).

The smoked beef packed in labeled, clean, plastic containers were stored in the Animal Product laboratory. Proximate, organoleptic and microbial analyses were carried out.

3.4 Sample Preparation for Suya

The muscles for this study were excised from the carcass of a three (3) years old White Fulani bull weighing 350 kg. The slaughtered animal was conventionally skinned and cut into wholesale cuts while the muscles needed were carefully removed. Muscles used are shown in Table 3.1.

Table 3.1 Round Muscles with their common names

Samples	**Muscle in the Round**	**Hausa Names**
Muscle 1	Rectus femoris	Jigiya
Muscle 2	Semi-Tendinosus	Farin Makari
Muscle 3	Biceps Femoris	Katara
Muscle 4	Semi Membranosus	Bishi
Muscle 5	Vastus lateralis	Tuwon kundu

The muscles were trimmed of all visible bones and connective tissues. Muscles were sliced into thin sheets of 0.15-0.30 mm thickness and length of 5-9 cm.

3.4:1 Ingredient Preparations

The spices used were purchased individually from specialized spice market in Sabo Zaria. The spices/additives include; Curry, Red pepper, Maggi seasoning, Onga spice, salt and Groundnut oil. All the spices were mixed together in a specific proportion as shown in Table 3.2

Table 3.2 Type and levels of ingredients used for the beef suya production

Ingredient Constituents	**Hausa Names**	**Proportion by Weight (g)**	**Percent Proportion in Mixture (%)**
Curry powder	Curry	5	1.10
Dried red pepper	Barkonu	169	37.06
Maggi Cube	Maggi	92	20.18
Onga spice	Onga	30	6.58
Salt	Gisiri	160	35.09
Total		456	100.01

3.4:2 Preparation of Suya

The tin sheets of beef from various muscles were inserted into thin sticks about 30 cm long. A total of 40 sticks of beef samples weighing 33.67- 43.16 were prepared from each muscle type. Sticks of Suya made from each muscle type (treatment), were labeled for easy identification. The average weight of ingredient per stick meat was measured after proper coating with the ingredient. Five to ten mills of groundnut oil was sprinkled on each suya sample prior to roasting.

3.4:4 Roasting Process

The labeled suya samples were arranged around a glowing, smokeless fire. The distance of the stick meat from the fire point was 21.96 - 23.3 cm. Suya samples were allowed to stay by the fire for 20 minutes with intermittent turning of the samples. Additional groundnut oil was sprinkled on the meat while roasting continued. The weight of each suya was recorded before and after roasting and used in calculating the percentage loss and the product yield. Samples for the determination of the physical, chemical properties, and microbial analysis of the suya were taken to the Animal Product Laboratory for analysis.

3.5 Sensory Evaluation

Smoked-cooked beef from round and suya samples were each cut into bites sample sizes and served in plates to a twenty member semi-trained panelists. The organoleptic parameters that were evaluated include appearance, taste, odour, texture and provision for a score on overall acceptability. A 5 point hedonic scale was used with a score of 5 indicating 'extremely acceptable', 4 'very acceptable', 3 'acceptable', 2 'fairly acceptable', 1 'not acceptable'. A score below 2 was considered not acceptable. The meat products were coded with numbers of 2 digits indicating no information about the samples to avoid bias in preferred treatments. The panelists received each sample separately, rinsing their mouth in-between samples.

3.6 Physical Composition of smoked beef and suya samples

3.6:1 Determination of Percentage Cooking Loss

Percentage cooking loss was determined by evaluating the differences in weight of initial sample from cooked divided by the weight before cooking multiplied by 100.

$$\% \text{ cooking loss} = \frac{\text{initial sample weight} - \text{cooked sample weight}}{\text{initial sample weight}} \times 100$$

3.6:2 Determination of Moisture content

Percentage moisture content was determined by the air oven method using 10 grams of meat samples at 80^0C to a constant weight. The difference in weight before and after smoking divided by weight before smoking multiplied by 100 was recorded (AOAC, 1990).

$$\text{MC, (\%)} = \frac{\text{WFS} - \text{WSS}}{\text{WFS}} \times 100$$

Where MC = Moisture Content (%),

WSS = Weight of Smoked Sample and WFS = Weight of Fresh Sample

3.6:3 Determination of Thermal shortening

This was carried out according to the procedure described by Mahendraker *et al.,* (1998). Cores taken from beef round and length measured prior to broiling in a fire stand for 10 min. After broiling, the beef cut was allowed to cool to room temperature and the length measured again with the difference in length expressed as percentage thermal shortening.

Thermal shortening (%) = (initial length – final length) × 100

3.6:4 Determination of Water Holding Capacity

Percentage water holding capacity was determined following a slightly modified method of Suzuki *et al*., (1997). Intact samples (10 x 10 x 5 mm) were weighed individually from the 5 muscle types on two filter papers and pressed for a minute, using a 10 kg weight.The amount of water released from the sample was measured indirectly by measuring the area of the filter paper wetted relative to the area of pressed sample. The water holding capacity (WHC) of the meat was calculated using the formulae developed by Suzuki et al., (1997)

$$\mathrm{WHC} = \frac{100 - (\mathrm{Ar} - \mathrm{Am}) \times 9.47}{\mathrm{Wm} \times \mathrm{Mo}} \times 100$$

Where Ar = Area of water released from meat (cm^2)

Am = Area of meat sample (cm^2)

Wm = Weight of meat in mg

Mo = Moisture content of meat %

9.47 is a constant factor.

3.6:5 pH Determination of smoked meat and suya

The pH of fresh beef and meat products were determined according to the method described by Bendall (1973). pH was measured in an aqueous extract from 1g of the dried samples homogenized in 10 ml distill water. The pH was measured using a Checker pH meter.

3.7 Proximate composition of smoked beef and suya

Proximate analyses were carried out using AOAC (1990) methods which include protein determination using Kjeldahl, fat extraction via Soxhlet, crude fiber determination using digestion with sulphuric acid, moisture determination by drying the sample for 16-18 hours at 100-102°C in an oven and mineral, by ashing the sample at 550°C for 9 hours in a furnace oven at the Animal Science Departmental Laboratory of ABU.

3.8 Microbial Analysis

3.8.1 Materials

The following bacteriological media were used; Modified tryptone soya broth, Sorbitol MaConkey agar, Nutrient agar, Triple Sugar Iron agar, Urea agar, Methyle Red Vosges Proskauer, Buffered peptone water, Eosine Methylen Blue, Sulphide Indole Mortility agar, Simmon Citrate agar, Potatoe Dextrose agar, Sabouraud Dextrose agar. All media used were of Oxoid product, others include Streptomycin powder and Injection water.

Laboratory equipments such as Stomacher laborotary blender (SWARD, London), Distilled water, Sterile Swab sticks, Cotton wool, Bijour bottles, Disposable Petri dishes, Pasteur pipettes, Polythene bags, Aluminum Foil, Electric Autoclave, Hot air ovum, Refrigerator, Test tubes, Conical flasks, Measuring cylinder, Bunsen burner, Wire loop and pin, Electric incubator, needle and Syringes and Electric weighing balance.

3.8.2 Preparation of Media for Fungi Isolation

Nutrient agar, Potato Dextrose Agar and MaConkey agar media were prepared according to the manufacturers' instruction.

3.8.3 Isolation of Fungi

The associated fungi were isolated using a standard pour plate technique. Ten grams of the dried meat products were homogenized in sterile distilled water. One millilitre of the homogenate was decimally diluted and 1 ml of selected dilution (10^{-4}) was plated in duplicate on a sterile Sabouraud Dextrose agar containing 1% streptomycin. Inoculated plates were incubated at 28 ± 2°C for 5-7 days. Discrete colonies were isolated in pure culture by sub-culturing the cells.

3.8.4 Characterizations of Fungal Isolates

The fungal isolates were identified based on their colonial morphology and microscopic examination of each fungal culture made in the vegetative and sporulation stages as recommended by Frazier and Westholf (1998). Characterization such as size, shape and septation of hyphae were also made. A clean slide with a drop of lactophenol blue in its centre was used for microscopic examination. A portion of the mycelium at the edge of the colony at growing edge/sporulating head was taken and placed in the drop of lactophenol blue using a flamed inoculating wire. It was then leased and covered with cover slip. The slide was mounted in the microscope, focused and observed at X40 objective lens. Appropriate observations were made.

3.8.5 Bacterial Counts (Coliform and Total Viable Counts)

25g/225ml of 0.1% peptone water and 5g of sample were weighed out using aseptic technique, and homogenized in enrichment medium to obtain 10^{-1} dilution. 10^{-1} bacterial suspension was serially diluted to 10^{-4}. 0.5 ml of each set pipetted onto the surfaces of two plates; Mac Conkey

for coliforms and Nutrient agar for Total viable count and immediately spread out with a sterile glass spreader i.e. using a standard pour plate technique. The Total viable count was calculated from the average colony count/plate after incubation. Microbial load guide in animal food product, according to Wilson *et al.,* (1991) is shown in table 3.3

Table 3.3 Standard Microbial load specification on animal food product.

Grades	TVC (total viable count)/g at 30°C	Description
I	<½ million	Satisfactory
II	½ million to <10 million	Passable
III	10 million and more	Unsatisfactory

Source: Wilson *et al*., (1991).

3.8.6 Bacteria Isolation by Selective Plating

The isolation was conducted according to the procedure of ISO, (2001). Ten grams of the dried meat samples were homogenized in 90ml of 1% buffered peptone water (selenite broth for *salmonella*) using a stomacher in sterilepolythene bag and incubated at 37^0C for 18-24 hours. Also, 1 ml of the homogenate was dispensed in 9 ml of sterile distilled water in a sterile test tube to give a 10^{-1} dilution. Further decimal dilutions were made until 10^{-4} dilution was obtained and incubated at 37^0c for 24hours. One millilitre from 10^{-4} dilution was taken to be inoculated by streaking on Eosin methylene blue (EMB) for E.*coli*, Xylose lysine deoxycholate agar (XLD) for *Salmonella* and Baird Parker Agar (BPA) for *Staphylococcus* isolation. Each sample was treated according to the protocol described by Lejeune *et al*., (2004). The plates were incubated at 37^0C for 24-48 hours for bacterial isolation.

3.8.7 Colony Identification

E.*coli* on EMB gives a distinctive metallic green sheen (due to the metachromatic properties of the dye) also noting that some citrobacter and enterobacter will also react this way on

EMB.*Salmonella* on XLD shows red colonies,some with black centers. *Staphylococcus on* Baird Parker Agar shows black colonies with clear zones produced around them.

Positive colonies were selected from each plate out of which colonies were sub-cultured. Pure (suggestive) cultures were inoculated and stored on nutrient agar slant and refrigerated for further biochemical analysis.

3.8.8 Biochemical Testing

These tests were carried out as described in the methodology of Khandaghi *et al*., (2010).

3.8:8:1 Triple Sugar Iron Agar Test (TSI)

In this test, the Triple Sugar Iron Agar was prepared according to the manufacturer's instruction and inoculated with the isolates both on the butt and the slant by stabbing followed by incubation at 37°C for 24-48 hours. It was then observed for hydrogen sulfide production (which is indicated by a black precipitate at the butt of the tube) and carbohydrate fermentation (indicated by gas production and colour change). Sulfide production and blackening of the medium at the interface of bottom and slant indicate presence of salmonella.

3.8:8:2 Indole Test

The pure isolates were grown in 5ml of tryptic soya broth and incubated at 37^0c for 24hrs. After which three drops of Kovacs indole reagent was added and shaken gently. After one minute, a positive reaction indicated by the development of a red/pink color in the reagent layer above the broth was indicative of *Escherichia coli,* a negative result appears yellow i.e. indicative of *salmonella sp.*

3.8:8:3 Methyl Red-Vogesproskauer Test

Pure isolates of the non-sorbitol fermenters were inoculated into 5ml of MR-VP broth and incubated for 48hrs at 35^0C. After incubation, about 1ml of the broth was transferred to a small serological tube followed by the addition of 2-3 drops of methyl red and the color on the surface of the medium read immediately. A red coloration on addition of the indicator signifies

a positive methyl red test. To the rest of the broth in the original tube, 5 drops of 40% potassium hydroxide (KOH) was added followed by 5 drops of 5% of alcoholic (ethanol) alpha-Naphthol and shaken. The cap of the tube was loosened and placed in a sloping position. The development of a red color starting from the liquid-air interface within 1hour indicates a positive test. *Escherichia coli* are reported to be Methyl red positive with an orange to red coloration and Voges-Proskauer negative with no coloration (Harrigan, 2000). Sallmonella is also Voges-Proskauer negative.

3.8:8:4 Citrate Utilization test

In this test a needle was used to pick a single isolated colony and streaking was done lightly on the surface of the Simmon's citrate agar slant which contains a pH indicator (Bromothymol blue) in a bijou bottle (whose screw cap would be placed loosely) and incubated at 35^0C for 24hours. At neutral pH as a result of organism present not utilizing citrate a green coloration of the indicator observed indicates a negative test. *Escherichia coli* are reported to be distinctively citrate negative (MacFaddin, 2000; Reddy, 2007), Salmonella is equally negative in citrate utilization test (Reddy, 2007).

3.9 Statistical Analysis

All data obtained were subjected to analysis of variance (ANOVA), and significant means separated using the Duncan's Multiple Range (DMR) test. The SAS computer package was used for all statistical analysis (SAS, 1999).

The model equation for the organoleptic properties was;

$Y_{ijkl} = \mu + M_i + E_{ijk}$

Y_{ijkl} = records of organoleptic properties

μ = overall mean

M_i = effect of the i^{th} smoking material on beef quality

E_{ijk} = random effect

CHAPTER 4

4.0 RESULTS:

4.1.1 Proximate Composition of Smoked Beef

Table 4.1 shows the proximate composition of smoked beef. Percentage Dry Matter content was highest in Meat smoked using coconut shell having 64.04% while Meat smoked with Acacia, Eucalyptus and Neem had values of 57.15, 55.25 and 50.37 %, respectively. The result for Crude Protein ranged between 40.93 - 43.87 %, with meat smoked using Neem wood having the highest value of 43.87. Values obtained for Fat extraction from Soxhlet equipment showed that Meat smoked with Coconut shell had the highest value of 7.98 %, while the least was observed in Meat smoked with Eucalyptus having 5.89 %, Meat smoked using Neem and Coconut had values of 6.77 % and 7.22 % respectively. The mineral composition showed that ash was highest in Meat smoked using Acacia (5.03 %), while Meat smoked with Neem, Coconut shell and Eucalyptus had values of 4.77, 2.27 and 2.76 %, respectively. Percentage nitrogen extract values are 44.13, 45.73, 40.47 and 41.13 for Meat smoked with Coconut shell, Eucalyptus, Neem and Acacia, respectivley. The Moisture content ranged between 31 - 40%, with Meat smoked using Acacia having higest value (40.35 %) while Meat smoked with Neem, Eucalyptus and Coconut shell had values of 39.02, 37.15 and 31.79 %, respectively.

4.1:2 Proximate composition of Suya produced from different muscles

Table 4.2 shows the Proximate composition of Suya from various round muscles i.e. muscle 1 (*Rectus femoris*), muscle 2 (*Semi-tendinosus*), muscle 3 *(Biceps Femoris*), muscle 4 *(Semi Membranosus) and* muscle 5 *(Vastus lateralis).* Suya produced from *Biceps Femoris* had the highest dry matter content (60.21 %) and the least was recorded in suya from *Rectus femoris* (39.58 %), *Semi-tendinosus*, *semi- membranosus* and *Vastus Lateralis* had dry matter values of 50.15, 41.91 and 43.61 %, respectively. The higest percentage crude protein was recorded in suya produced from *Biceps Femoris* having a value of 45.31, *Semi membranosus*, *Semi-*

tendinosus, *Rectus femoris* and *Vastus lateralis* had values of 41.91, 44.50, 39.06 and 38.37 %, respectively. Values obtained for percentage ash content was higest in suya produced from *Semi-tendinosus* (5.53 %), followed by *Vastus lateralis* (4.77 %), *Semi- membranosus* (4.48 %) *Biceps femoris* (4.04 %) and *Rectus femoris* (3.38 %). Nitrogen free extract values were 43.9, 37.03, 38.63, 38.43 and 43.44 %, obtained for suya from *Rectus femoris*, *Semi-tendinosus*, *Biceps Femoris*, *Semi Membranosus* and *Vastus lateralis*, respectively.

4.2 Mean Sensory Score of Colour, Juciness, Taste, Smoky Flavour, Texture and Overall Acceptability

4.2:1 Organoleptic assessment of beef smoked using different plant materials

Table 4.3 shows the mean sensory score of Colour, Juciness, Taste, Smoky flavour, Texture and Overall acceptability for meat smoked with *Acacia raddiana*, *Eucalyptus camaldutensis*, Neem and coconut shell. Mean values obtained for colour showed that Meat smoked with Acacia and Eucalyptus had the higest values of 2.60 each which were statistically similar to Meat smoked with Neem (2.35), but differed from Me at smoked using Cocunut shell (1.57). Values obtained for Juiciness showed highest mean in Meat smoked with Acacia (2.85) which is statistically same with Meat smoked using Neem (2.65) and Eucalyptus (2.30) but differed significantly ($p<0.05$) from Meat smoked using Coconut shell (1.40). Taste was higest in Meat smoked using Neem (2.85) which was statistically similar Meat smoked using Acacia (2.30) was statistically similar to meat smoked using eucalyptus (2.05) and Coconut shell (1.50). Smoky flavour shows difference across treatment. The lowest value for smoky flavor was observed in Meat smoked using Coconut shell (2.00), the highest mean was given by Meat smoked using acacia (2.85%). This was statistically similar to Meat smoked with Neem (2.65) and Eucalyptus (2.35) which was similar to Meat smoked using Coconut shell with 2.00. Values of 3.50, 3.20, 2.96 and 2.85 obtained for Meat smoked using Coconut shell, Eucalyptus, Neem and Acacia respectively were not significantly different ($P<0.05$). Values obtained for overall acceptability were not significant ($P<0.05$).

Table 4.1: Proximate and mineral composition of smoked beef samples

Samples	%D.M	%C.P	% Ether extract	%Ash	%NFE	% Moisture
MA	57.15	41.87	7.22	5.03	41.13	40.35
ME	55.25	41.31	5.89	2.27	45.73	37.15
MN	50.37	43.87	6.77	4.17	40.47	39.02
MC	64.04	40.93	7.98	2.76	44.13	31.79

Results were mean of triplicate sample readings.

Key MA: Meat smoked using *A. raddiana*, ME: Meat smoked using *E. camaldutensis*
MN: Meat smoked using Neem, MC: Meat smoked using Coconut shell

Table 4.2: Proximate and mineral composition of Suya muscles

Suya Samples	%D.M	%C.P	%Ether Extract	%Ash	%NFE
Rectus femoris	39.58	39.06	6.87	3.38	43.9
Semi-tendinosus	50.15	44.50	7.65	5.53	37.03
Biceps Femoris	60.21	45.31	7.39	4.04	38.63
Semi Membranosus	41.91	44.50	8.03	4.48	38.43
Vastus lateralis	43.61	38.37	8.23	4.77	43.44

Table 4.3 Organoleptic properties of Smoked beef samples

Samples	Colour	Juiciness	Taste	Smoky flavor	Texture	Overall acceptability
MA	2.60^{a}	2.85^{a}	2.30^{ab}	2.85^{a}	2.85	3.30
ME	2.60^{a}	2.30^{a}	2.05^{bc}	2.35^{ab}	3.20	2.60
MN	2.35^{ab}	2.65^{a}	2.85^{a}	2.65^{ab}	2.96	3.15
MC	1.57^{b}	1.40^{b}	1.50^{c}	2.00^{b}	3.50	2.50
SEM	0.12	0.12	0.12	0.13	0.13	0.14

Means in the same Colum with different superscript are significantly (P>0.05) different.

4.2:2 Organoleptic Assessments of Suya Samples

Table 4.4 shows the mean sensory score of colour, juciness, taste, smoky flavour, texture and overall acceptability for suya produced from various muscles (*Rectus femoris*, *Semi-tendinosus*, *Biceps Femoris*, *Semi Membranosus* and *Vastus lateralis*). Mean values obtained for colour showed that Suya from *biceps femoris* had the highest mean of 4.21 which was statistically ($P > 0.05$) thesame with Suya produced from *Rectus femoris* (3.53). Suya from *Rectus femoris* and *Biceps femoris* were statistically similar to suya from *vastus lateralis* (3.32) and *Semi-tendinosus* (2.74). The least value was recorded in suya from *Semi membranosus* (2.47). Mean values for Juiciness had the highest value recorded in Suya produced from *Vastus lateralis* (3.58), which was statistically same with Suya from *Rectus femoris* (3.47). Suya from *Rectus femoris* was statistically similar to suya produced from *biceps femoris* and *Semi-tendinosus* (3.37 and 2.84, respectively). Mean values for juiciness in beef suya from *Biceps femoris* and *semi-tendinosus* are statistically similar to *semi-membranosus* (2.63) which had the least, suya from *Rectus femoris* and *Vastus lateralis* differed significantly ($p<0.05$) from *Semi-membranosus*. Mean texture ratings had highest values Suya from *Semi-membranosus* and *Rectus femoris* (3.91 and 3.54, respectively), which are statistically similar to values obtained form *Vastus lateralis* and *Biceps fermoris* (2.95 and 3.00 respectively). Suya from *Vastus lateralis* and *Biceps fermoris* are statistically similar to suya from *Semi*-tendinosus (2.90). Mean values for smoky flavour were not statistically significant having values of 3.16, 3.00, 3.26, 3.00 and 2.84, respectively. Similarly, mean values for Saltiness were not statistically significant.

4.3 Physical Parameters/Changes of Final products

4.3:1 Physical Parameters of Smoked Beef

Table 4.5 shows the physical parameters of smoked beef. The mean values obtained for pH had the highest pH value in beef smoked with coconut shell (6.43). Beef smoke with

Eucalyptus and Acacia were statistically the same (6.10 and 6.00, respectively), with the lowest pH value recorded in beef smoked with neem wood (5.80). Percentage cooking loss for smoked beef was highest in beef smoked with Acacia (40.00 %), which was statistically same with beef smoked with Neem wood (39.00 %). The lowest value for percentage cooking loss was recorded in beef smoked with Eucalyptus (23. 15 %). Values obtained for percentage Thermal Shortening were statistically not significant (P >0.05). Water holding capacity of smoke beef samples ranges from 10.93 - 13.69 with beef smoked with coconut shell having the highest value (13.69).

4.3:2 Physical parameters of Suya

Table 4.6 shows the Physical changes in the final beef product (Suya). Values for meat to ingredient ratio for suya samples were; 18:1, 14:1, 14:1, 13:1 and 13:1, across samples (i.e. Suya produced from *Vastus lateralis, Semi-tendinosus, Biceps femoris, Rectus femoris* and *Semi membranosus,* respectively). Suya produced from *vastus lateralis* muscle had the lowest ingredient absorption. *Vastus lateralis* recorded the higest percentage weight loss after roasting (41.66). Percentage weight loss for beef suya produced from *Rectus femoris, Biceps femoris, Semi-tendinosus* and *Semi membranosus* had values of 33.98 %, 20.27 %, 18.1 % and 15.98 %, respectively. pH values ranged between 5.70 to 6.50 across samples. Percentage water content was higest in suya made from *Vastus lateralis* (25.05 %), Suya produced from *Semi membranosus, Rectus femoris, Semi-tendinosus* and *Biceps femoris* had mean values of 24.35 %, 22.04 %, 18.98 % and 18.09 %, respectively. Percentage product yield values are 84.02 %, 82.81 %, 79 %, 74.63 % and 70.59 % (i.e. Suya produced from *Semi membranosus, Semi-tendinosus, Biceps femoris, Rectus femoris* and *Vastus lateralis,* respectively).

Table 4.4: Organoleptic properties of Suya samples

Samples	Colour	Juiciness	Texture	Smoky flavor	Saltiness	Overall acceptability
Rectus femoris	3.53[a]	3.47[a]	3.54[a]	3.16	2.42	3.95[ab]
Semi-tendinosus	2.74[ab]	2.84[ab]	2.90[b]	3.00	2.32	3.42[b]
Biceps Femoris	4.21[a]	3.37[ab]	3.00[ab]	3.26	2.42	4.26[a]
Semi Membranosus	2.47[b]	2.63[b]	3.91[a]	3.00	2.21	3.95[ab]
Vastus lateralis	3.32[ab]	3.58[a]	2.95[ab]	2.84	2.16	3.90[ab]
SEM	0.27	0.23	0.70	0.17	0.57	0.20

Means in the same row with different superscripts are significantly different (P<0.05)

*Rated on a five-point hedonic scale. Higher value indicates higher preference.

Table 4.5: The effect of using different plant materials for smoking beef on some of the Physical Parameters

Samples	pH	Cooking loss %	Thermal shortening %	W.H.C %
MA	6.00[b]	40.00[a]	6.00	10.93[b]
ME	6.10[b]	23.15[c]	5.57	10.93[b]
MN	5.80[c]	39.00[a]	6.57	12.44[ab]
MC	6.43[a]	32.00[b]	6.12	13.69[a]
SEM	0.05	0.57	0.34	0.57

Key MA: Meat smoked using *A. raddiana*, ME: Meat smoked using *E. camaldutensis*
MN: Meat smoked using Neem, MC: Meat smoked using Coconut shell

Table 4.6: Physical Changes in the Final Product (Suya) produced from various muscles

Parameters	*Rectus femoris*	*Semi-tendinosus*	*Biceps femoris*	*Semi membranosus*	*Vastus lateralis*
Weight of stick (g)	5.0	5.0	5.2	5.3	5.0
Weight of meat (g)	58.70	72.01	58.59	57.70	35.98
Weight of ingredient (g)	4.39	4.99	4.13	4.20	1.98
Weight of suya before roasting (g)	63.09	77	62.72	61.9	34
Meat: ingredient ratio	13.4:1	14.2:1	14.2:1	13.7:1	18:1
Weight after roasting (g)	47.09	63.76	50.01	52.01	24
Percentage weight loss	33.98	18.1	20.27	15.98	41.66
pH	5.70	6.50	6.20	5.80	6.00
Water Holding Capacity (%)	22.04	18.98	18.09	24.35	25.05
Product yield (%)	74.63	82.81	79.00	84.02	70.59

5 – 10 ml of groundnut oil was added to each stick of meat during roasting

4.4 Total Viable Counts, Coli-form Counts

4.4:1 Total Aerobic Plate Counts, Viable Counts and Coli-Form Counts of Smoked Beef

The results obtained (Table 4.7) showed that the dried beef samples were contaminated with microorganisms despite the dried and hard texture of the meat. Total viable counts/Total aerobic plate count, coli-form counts were all within satisfactory limit. Total aerobic plate count on Nutrient agar was highest in beef smoked with Acacia wood (1.47×10^6), Total aerobic plate count for beef smoked with Coconut shell and Eucalyptus on Nutrient Agar were 29×10^4 and 19×10^4, and the lowest value was recorded in beef smoked with Neem wood (8×10^4). Total viable count on MacConkey agar was highest in beef smoked with coconut shell (36×10^4), beef smoked with Acacia and Eucalyptus had values of 23×10^4 and 19×10^4, respectively and the lowest value was recorded in beef smoked with Neem wood. No Coli-form count was observed in beef smoked with Eucalyptus and Neem wood in dilution of 10^4 on Eosin Methylene Blue Agar. However, beef smoked with Acacia wood and Coconut shell had 8×10^4 and 9×10^4, respectively.

4.4:2 Total Viable Counts/Total Aerobic Plate Count, Coli-Form Counts Of Suya

Table 4.8 shows the Total viable counts/Total aerobic plate count, coli-form counts of suya samples. Suya samples were contaminated with microorganism though were within satisfactory limit. Values recorded for Total aerobic plate counts on Nutrient agar was higher in suya from *Vastus lateralis* (2.98×10^6) and *rectus femoris* muscle (2.15×10^6), suya samples from *semi-tendiosus*, *biceps femoris* and *semi membranosus* had 56×10^4, 51×10^4 and 42×10^4, respectively. Results for Total viable count on MacConkey agar had higher values observed in suya from *semi-tendinosus* and *vastus lateralis* muscles (1.32×10^6 and 1.20×10^6, respectively), values for suya from *rectus femoris, biceps femoris* and *semi membranosus* are 79×10^4, 93×10^4

and 73×10^4, respectively. There was no Coli-Form unit observed in suya from *rectus femoris* and *biceps femoris* muscles on Eosin Methylene Blue, suya from *vastus lateralis, semi membranosus* and *semi tendinosus* had values of 4.9×10^4, 3×10^4 and 1×10^4, respectively.

4.5 Biochemical Test

4.5:1 Biochemical Test for Salmonella in Smoked Beef Samples

Table 4.9 shows the biochemical test results for salmonella on smoked beef samples. Beef smoked with eucalyptus was postive for salmonella while the other samples were not (i.e. negative).

4.5.2 Biochemical Test Result of Salmonella on Suya Samples

Table 4.10 shows the biochemical test results for salmonella on suya samples. Muscle 5 (Vastus lateralis) was postive for salmonella while the other samples were not (i.e. negative).

Table 4.7: Total aerobic plate count**,** Total viable counts, coli-form counts (Smoked beef)

Sample	TVC CFU/ml	Description	CFC Count CFU/ml	Description	TAPC CFU/ml	Description
MA	1.47×10^6	Passable	23×10^4	Satisfactory	8×10^4	Satisfactory
ME	19×10^4	Satisfactory	19×10^4	Satisfactory	-	Satisfactory
MN	8×10^4	Satisfactory	12×10^4	Satisfactory	-	Satisfactory
MC	29×10^4	Satisfactory	36×10^4	Satisfactory	9×10^4	Satisfactory

Key= MA: Meat smoked using *A. raddiana*, ME: Meat smoked using *E. camaldutensis*
MN: Meat smoked using Neem, MC: Meat smoked using Coconut shell,
TVC: Total Viable count on Nutrient Agar, CFC:Coli-form Count on MaConkey Agar
TAPC: Total aerobic plate count on Eosin Methylene Blue.

Table 4.8: Total Aerobic Plate Count, Total Viable Counts, Coli-Form Counts of Suya produced from various beef round muscles

Sample	TAPC. On NA CFU/ml	Description	TVC. On MCA(LF) CFU/ml	Description	EMB(GMS) CFU/ml	Description
Rectus femoris	2.15×10^6	Satisfactory	79×10^4	Passable	-	Satisfactory
Semi-tendinosus	56×10^4	Passable	1.32×10^6	Passable	1×10^4	Satisfactory
Biceps Femoris	51×10^4	Passable	93×10^4	Passable	-	Satisfactory
Semi Membranosus	42×10^4	Passable	73×10^4	Passable	3×10^4	Satisfactory
Vastus lateralis	2.98×10^6	Satisfactory	1.20×10^6	Passable	4.9×10^4	Satisfactory

TAPC: Total aerobic plate count, TVC: Total viable counts, NA: Nutrient Agar, MCA: MacConkey Agar, EMB: Eosin methylene blue

Table 4.9: Biochemical Test Result of Salmonella in Smoked Beef Samples

Samples	*TSI*	*Urea*	*Citrate*	*Indole*	*H_2S*	*Motility*	*Confirmed org.*
MA	a/a	+	+	-	+	+	ND
ME	al/a	-	+	-	+	+	Salmonella
MN	a/a	+	+	-	+	+	ND
MC	a/a	+	+	+	+	+	ND

ND: Not Determined, MA: Meat smoked using *A. raddiana,* ME: Meat smoked using *E. camaldutensis*, MN: Meat smoked using Neem, MC: Meat smoked using Coconut shell

Table 4.10: Biochemical test result of salmonella in suya samples

Samples	**TSI**	**Urea**	**Citrate**	**Indole**	**H_2S**	**Motility**	**Confirmed org.**
Rectus femoris	al/a	+	+	-	+	+	ND
Semi-tendinosus	a/a	+	+	-	-	+	ND
Biceps Femoris	a/a	+	+	+	+	+	ND
Semi Membranosus	al/a +g	+	-	+	-	+	ND
Vastus lateralis	a/a	-	+	-	+	+	Salmonella

ND: Not Determined

4.5.3 Biochemical Test Result of *E.coli* on Smoked Beef

Table 4.11 shows the biochemical test results for *Eschericha coli* on smoked beef samples. MC (beef smoked with coconut shell) was postive for *E-coli* while the other samples were not (i.e. negative).

4.5.4 Biochemical Test Result of *Eschericha Coli* on Suya Samples

Table 4.12 shows the biochemical test results for *Eschericha coli* on suya samples. Muscle 5 (*Vastus lateralis*) was postive for *E-coli*, while the other samples were not (i.e. negative).

4.6 Xerophilic Fungi Isolated

4.6.1 Moulds associated with Smoked beef

Table 4.13 shows the presence of fungi in the sample, *A. niger* was identified in beef smoked with Acacia, Eucalyptus and Neem, *A. flavus* was identified in beef smoked with Neem and Coconut, *Penicillium spp* was identified in Beef smoked with Eucalyptus and Coconut shell. *Rhizoppus spp* was identified in beef smoked using eucalyptus, and *Candida spp* identified in beef smoked using acacia and coconut shell.

4.6.2 Moulds associated with suya

Table 4.14 shows the presence of fungi in the sample, *A. niger* was identified in Muscle4, and Muscle 5, *A. flavus* was identified in Muscle 3 and Muscle 5, *Penicillium spp* was identified in Muscle 1 and Muscle 4, *Rhizoppus spp* was identified in Muscle 2 and*Fusarium*spp identified in Muscle 3 Rectus femoris, Semi-tendinosus, Biceps Femoris, Semi Membranosus and Vastus lateralis.

Table 4.11: Biochemical Test Result of *E-Coli* in Smoked beef

Samples	***TSI***	***Urea***	***Citrate***	***Indole***	***MR***	***VP***	***H_2S***	***Mortility***	***Confirmed org.***
MA	a/a +g	+	-	+	+	-	+	+	ND
ME	al/a	+	+	+	+	-	+	+	ND
MN	a/a +g	+	+	+	-	+	+	+	ND
MC	a/a +g	-	-	+	+	-	-	+	E-coli

ND: Not Determined, MA: Meat smoked using *A. raddiana,* ME: Meat smoked using *E. camaldutensis*, MN: Meat smoked using Neem, MC: Meat smoked using Coconut shell.

Table 4.12: Biochemical Test Result of *E-Coli suya*

Samples	***TSI***	***Urea***	***Citrate***	***Indole***	***MR***	***VP***	***H_2S***	***Mortility***	***Confirmed org.***
Rectus femoris	a/a +g	+	+	+	-	+	-	+	ND
Semi-tendinosus	a/a	+	+	+	+	-	-	+	ND
Biceps Femoris	al/a	+	+	+	+	-	+	+	ND
Semi Membranosus	a/a	+	+	+	+	-	+	+	ND
Vastus lateralis	a/a	+	+	-	+	-	+	+	E-coli

ND: Not Determined

Table 4.13: Moulds associated with Smoked beef

Xerophilic Fungi isolated	***MA***	***ME***	***MN***	***MC***
Aspergillus niger	*+*	*+*	*+*	-
Aspergillus flavus	-	-	*+*	*+*
Penicillium spp	-	*+*	-	*+*
Rhizoppus spp	-	*+*	-	-
Candida spp	*+*	-	-	*+*

(+): isolated (-): not isolated, MA: Meat smoked using *Acacia raddiana,* ME: Meat smoked using *Eucalyptus camaldutensis*, MN: Meat smoked using Neem, MC: Meat smoked using Coconut shell

Table 4.14: Fungi found on suya samples produced from different muscles.

Fungi isolated	*Rectus femoris*	*Semi-tendinosus*	*Biceps Femoris*	*Semi Membranosus*	*Vastus lateralis.*
Rhizoppus spp	-	+	-	-	-
Aspergillus niger	-	-	-	+	+
Aspergillus flavus	-	-	+	-	+
Penicillium spp	+	-	-	+	-
Fusarium spp	-	-	+	-	-

(+): isolated (-): not isolated

Description of the microbes identified.

Xerophilic Fungi isolated	**Microbes description**
Apergillus flavus	Large bright green colonies with yellowish centuries, Sterigmata Uniseriate.
Aspergillus Niger	Black radiating colonies with large conidia heads was seen.
Penicillium spp.	Had distinct blue-green white colouration at first then fully coloured after conidial matured.
Fusarium spp.	This fungus was at first white in colony then it gradually became pale on hyphae or in the substrate.



Plate 1a: *Aspergillus Niger (black),*

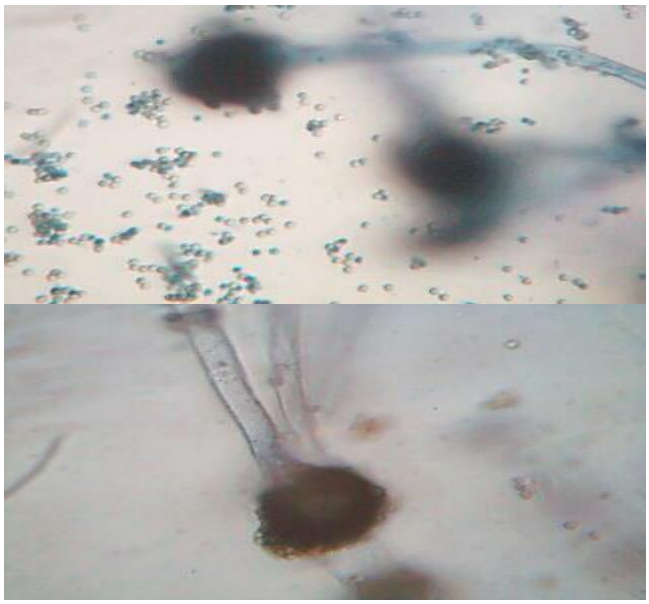
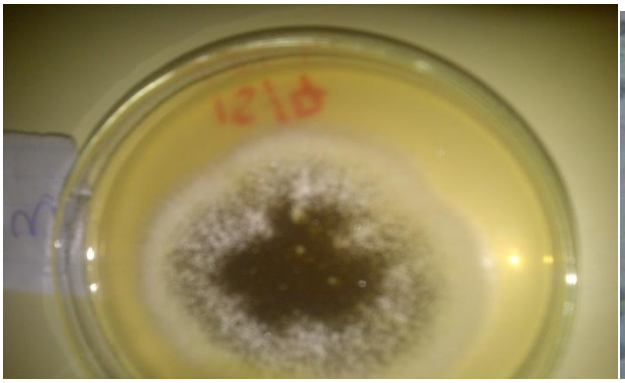


Plate 1b: Photomicrograph of *Aspergillus* spp**. (×1,000)**



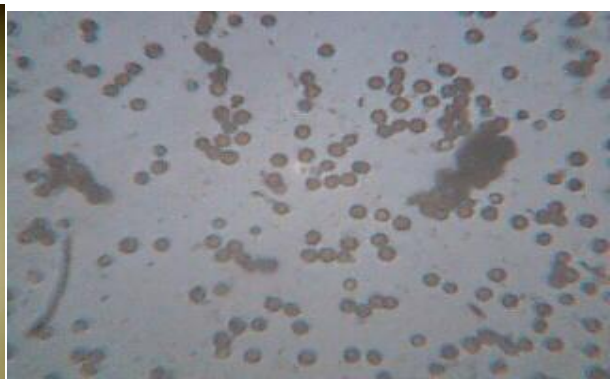
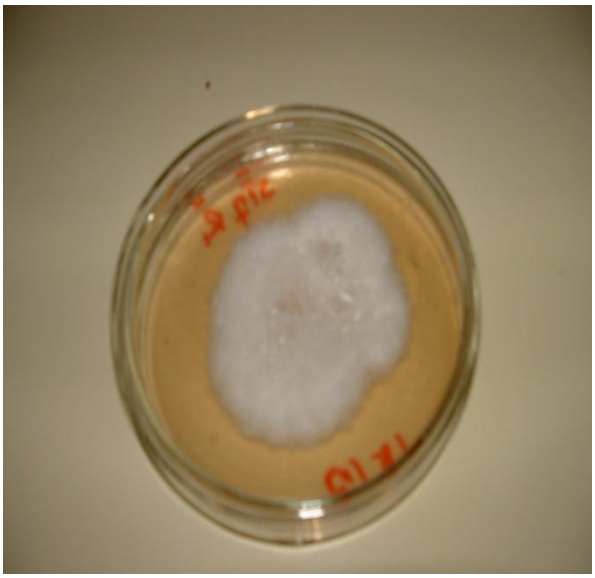


Plate 2a:*Aspergillus Niger* ***(brown)***

Plate 2b: Photomicrograph A. *Niger* spp.(×1,000)





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Plate 3a: *Candida spp.*

Plate 3b: Photomicrograph of *candida* spp. (×1,000)



Plate 4a: *Fusarium* spp.

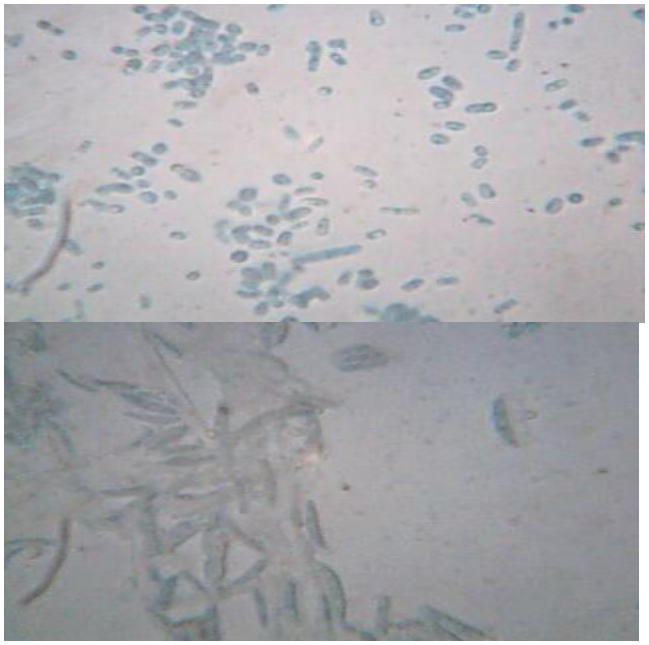


Plate 4 b: Photomicrograph of *Fusarium* spp **(**×1000**)**

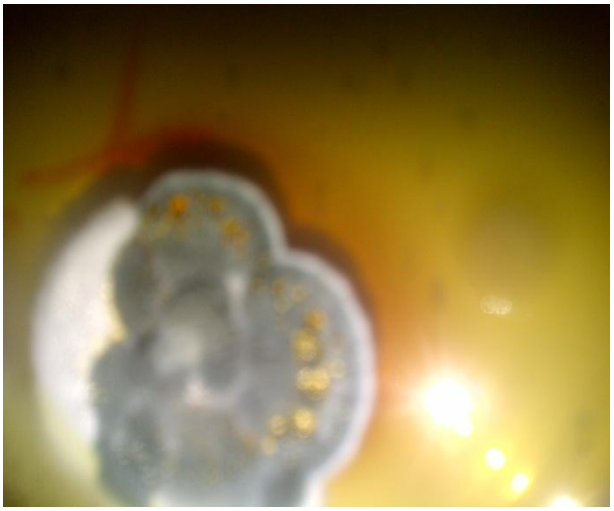


Plate 5a: *Penicillium* spp.



Plate 5b: Photomicrograph of *Penicillium* spp**. (**×1000**)**

CHAPTER 5

5.0 DISCUSSION

5.1 Proximate and mineral composition of smoked beef

The Percentage dry matter content was highest (64.04 %) in beef smoked with coconut shell followed by acacia, eucalyptus and neem, respectively (Table 4.1). There was an inverse relationship between percentage dry matter and Cooking Loss of the smoked meat samples. The inverse relationship observed in this experiment agreed with report by Fakolade, (2012) on proximate composition of Kundi from camel and three breeds of cattle. The Crude Protein value ranged between 40.93 - 43.87 %, with beef smoked with Neem having a higher value of 43.87 %. The high protein content observed in beef smoked with various wood fuel sources agrees with Egbunike and Okubanjo (2000) who reported that Intermediate Moisture Meats are low in moisture content, less bulky and have higher protein than raw protein equivalent. However, the production process has been reported to lead to the loss of some soluble protein (Mboung, 1999). The Ether Extract value ranged between 5.89–7.98 % which is comparable with 1.5 - 13.0% reported by Hedrick *et al*., (1994) for smoked meat product. The higher fat content observed in beef smoked with Acacia (7.98 %) could be attributed to the low moisture and fat content relationship as suggested by Fakolade (2012), due to the fact that they relate inversely. Fat is very important in flavour development of meat, as meat ages the fat deteriorates through microbial attack, tissue enzyme activity and oxidation of unsaturated bonds, this results in the development of bad odours and deterioration of taste. Unpleasant flavors in cooked beef were first noticeable at a level of 2 - 3% (as oleic acid) in extracted fat equivalent to 20 % ether extract observed by Pearson (1996). Moisture Content of smoked beef obtained, was within 30 – 40 %, equally reported by Fakolade and Omojola, (2008) for Kundi. The values were however higher than 8.2 % - 11.1 % observed for moisture of oven dried and sun dried Kilishi reported by Egbunike and Okubanjo (2000). The moisture content observed

for smoked beef after processing using acacia, eucalyptus, neem wood and coconut shell, was within 30 - 40%, which is quite low for microbial survival and activities in such medium. The values for percentage proximate composition of meat product from different smoke source appeared to be within ranges reported by other authors.

5.2 Proximate composition of Suya from various round muscles

Crude Protein values found in suya products were within the range of 40 – 45 %. Protein found in suya products were incomp arable (69.8 %) with those reported by Soniran and Okubanjo (2002) for protein content of meat cooked to three internal temperatures at 65^0C, 75^0C and 85^0C, respectively. Protein contents of beef suya samples in this study were equally observed to be greater than its fresh protein equivalent of 21.96 % for camel and 18.95% for fresh beef. The difference in protein content observed between suya from different muscles may be attributed to the type and activity of the muscle, the function and type of tissue fibre that makes up the muscle. The higer value observed in the protein content reported by other authors may be due to the groundnut cake added as ingredient which is high in protein. Fat extraction from Soxhlet equipment showed suya produced from *Vastus lateralis* had the highest value of 7.98 %, while the least was observed in suya from *Semi-tendinosus* having 5.89 %. Difference observed in fat content of suya from muscles may be due to the effect of nutrient concentration due to moisture loss or fat deposition in different tissues. Values obtained for Ether extract in this study (7.97-5.89 %) are slightly lower than 8.40 - 9.50 % reported by Apata e*t al.*, (2012). The higher values observed from other studies may be due to the application of excess groundnut oil to the suya sample to reduce mold infections.The mean ash content agreed with report by Fakolade and Omojola (2008), the authors found that ash content of meat increased with heat application. Torres *et al.*, (1994) suggested that relatively higher content observed (8.03 %) may be as a result of increase storage time of suya. The values for percentage

proximate composition of meat product (suya) from different round muscles appeared to be within ranges reported by other authors.

5.3 Mean sensory score for Colour, Juciness, Taste, Smoky flavour, Texture and Overall acceptability of smoked beef

There was no significant difference ($P<0.05$) in the overall acceptibilty of meat smoked using A. *raddiana*, E. *camaldutensis*, Neem and coconut shell when compared to A. *raddiana* as shown in Table 4.3. For all the organoleptic parameters, beef smoked with coconut shell as fuel wood recorded the lowest values except texture, although they were all above 2. The newer trees used, scored above 2 which is the limit of acceptability and competed well with *Acacia*, the conventional wood fuel. Result observed are comparable with study involving trials with *Acacia* tree species as fuel wood, good appearance and taste were recorded (Oduor-odute *et al.*, 2008 and 2010). Coconuts shells used to smoke catfish fillets in a separate study by Benjakul and Aroonrueng, (1999) gave acceptable attribute ratings comparable to what is found in this study.

Many factors influence the quality of smoked meat products including the properties of meat flesh, age at slaughter, sex of animal and factors involved in the smoking procedure such as wood type, composition of smoke, temperature, humidity, velocity and density of the smoke (Simko, 2005). Phenolic and carbonyl compounds contribute towards taste in smoked meat (Martinez *et al.*, 2007). Specific volatile compounds in particular phenolic compounds have been related to the different smoking techniques which directly influence the sensory characteristics of smoked meat. Organoleptic properties of smoked foods are decisively influenced by composition of the smoke and nature of wood involved. There is no agreement about which wood or mixture of woods imparts the preferred sensorial properties to smoked meat. The fact that there is no significant difference in sensory attributes between the various

wood types during smoking in this study suggests that E. *camaldutensis*, Neem and coconut shell can be used in meat smoking. However, there might be a negative impression towards beef smoked using Neem as wood fuel. This could be due to the influence in culinary habits and the bitter taste experienced after consumption of its liquid extract. The slight differences in taste observed between the wood types could be due to the different compounds in the various smokes interacting differently with components of the beef. Different wood sources give wood smokes that have distinctly different sensory properties. Kjällstrand and Petersson (2001), reported that reactions between the carbonyl compounds and proteins are mainly responsible for colour formation on smoked surfaces suggesting that smoke from *Acacia* and *Eucalyptus* had higher carbonyl compounds while the absorbed phenolic compounds are related to flavor and aroma of the smoked product.

5.4 Mean sensory score for organoleptic parameters of Suya

The muscle types used for suya affected the Juiciness ratings as shown in Table 4.4. The highest score was given to suya produced from *vastus lateralis* which had the highest water holding capacity. The observed relationship agrees with statement by Omojola *et al.* (2003) who reported that Juiciness is made up of two effects; the impression of moisture released during chewing and also the salivation produced by flavour factor. Meat juices play an important role in conveying the overall impression of palatability to the consumer. They contain many of the important flavour components and assist in the process of fragmenting and softening the meat during chewing (Hedrick *et al.,* 1994).

Tenderness ratings were highest in suya produced from *Rectus femoris* and *semi membranosus* having values of 3.54 and 3.91, respectively on a 5 point hedonic scale, tenderness ratings were comparable to 5.97 on a 9 point hedonic scale reported by Apata *et al.,* (2012). Variation exists

among muscles and differences may be due to the amount of connective tissue in the various cuts and amount of connective tissue present is due to the function of the muscle.

Cross *et al.,* (1996) as sited by Omojola (2008) reported that tenderness is considered as the most important trait in meat quality. It has also been identified as the most critical eating quality that determines whether consumers are repeat buyers. However it is worthy of note that as a result of the heat treatment and the use of spices which have tenderizing effect the degree of tenderness is affected when compared to fresh meat. Okubanjo (1990) as sited by Soniran and Okubanjo (2002) reported that most citizens of developing countries like Nigeria prefer less tender meat or meat product probably for longer chewability.

The result obtained for the Overall Acceptability indicate that the consumers have preference for suya from muscle 3 (*Biceps femoris*) which significantly ($P<0.05$) differed in value from suya obtained from other round muscles. It was reported (Anjaneyulu *et al.,* 2007) that most consumer's adjudge meat and meat products based first on colour and then flavour, which agreed with the relationship observed in this study

5.5 Physical Parameters of Smoked Beef

Water holding capacity, which is the ability of meat to retain its water during external forces such as cutting, heating, grinding and pressing, gave values that ranged between 11 – 15 % (Table 4.5) which is lower than values (59 %) reported by Fakolade (2012). This is important in meat processing as it influences the overall eating quality. Beef smoked with *Acacia* and *Eucalyptus* gave better water holding capacity (10.93 %). When meat is heated, protein denatures and coagulates causing spaces within the myofibril protein to decrease consequently. Thermal shortening ranged from 5.57 -6.57 % were not significantly ($p>0.05$) different. Thermal shortening also occurs as a result of structural changes caused by heating. There is a positive relationship between thermal shortening, WHC and product yield. Hence, the

percentage thermal shortening, water holding capacity and cooking loss were highest in beef smoked using *Acacia radiana* suggesting higher temperature produced by *Acacia raddiana* wood during the smoking. Tronberg (2005), reported that cooking induces structural changes and in turn decrease the water holding capacity of meat, thereby causing increase in shortening and rigidity, hence reduced moisture content. Cooking loss refers to the combination of liquid and soluble matter lost from the meat during cooking. Percentage Cooking loss was highest in beef smoked using *Acacia* and Neem (60 and 59 %). Heymann *et al.*, (1990), indicated that cooking loss is mostly water and could be caused by protein denaturation which allows less water to be entrapped within the protein structures held by capillary forces. Okubanjo *et al.*, (2003) said that water or cooking loss is of economic concern because it affects weight loss along the distributions chain. Values obtained in this study are incomparable with those reported by Fakolade (2012) for oven dried beef. The pH values for fresh beef were below the maximum accepted limit of 6.0 suggested by Pearson (1996) and sited by Fokalade (2012) for fresh meat, suggesting that the products were produced from well-nourished and rested stock. pH of smoked beef ranged from 5.80 to 6.50.

5.6 Physical changes in Suya

Water holding capacity refers to the ability of meat to retain its water during application of external forces (Hedrick *et al.,* 1994). Water holding capacity as shown in table 4.6 was highest in suya from *vastus lateralis* (25.05 %). It is important in meat processing as it influences the overall eating quality. Water holding capacity in this experiment was observed to have a direct relationship with other parameters such as the flavour, juiciness and tenderness which agrees with the report by Omojola (2008). The values of the water holding capacity (i.e. 18 – 25 %) in this study are slighty lower than values reported by Apata (2012), having water holding capacity of 21 – 35 % for suya samples. The slight difference observered in suya from the muscle types may be due to the type of connective tissues and activity of the muscles. Alonge

(1994) reported that Suya products with lower moisture content of about 30%-40% and a_w of 0.85 allow only the growth of fungi microbes. Suya from *Vastus lateralis* had the highest fat content (8.23 %) and the highest water holding capacity suggesting that leaner meat contain more protein and less fat, and since water is a component of protein (but not fat), a learner cut will contain more water.

Incidentally, weight of ingredient uptake was lowest (1.98 g) in suya from *vastus lateralis* which had the highest water holding capacity, this trend was also recorded in report by Omojola (2008). The amount of water loss when high in muscle, translates to a higher weep and a greater medium for the uptake of more ingredients.

The product yield was lowest in suya from *Vastus lateralis* (70 %), indicating a good yield from all muscles. Offer (1984) as sited by Fakolade (2012), summarized the structural changes occurring in cooking or drying processes during meat preservation processes as a traverse shrinkage to the fibre axis occurs mainly at 40-60 °C, this widens' the gap already presents at rigor between the meat fibres and their surrounding endomysium. At 60-70 °C the connective tissue networks and the muscle fibres co'operatively shrink longitudinally. Tonberg (2005), reported that the extent of shrinkage increases with temperature and causes a great water loss, hence the direct relationships between products yield and cooking loss observed in this study.

5.7 Total Viable Counts, Coli-Form Counts of Smoked beef

Total viable counts, coli-form counts of smoked beef from all treatment (Table 4.7) are within control limits (Wilson *et al.*, 1991), probably due to lower moisture contents, pH, anti-microbial properties of the various smoke and water holding capacity of smoked samples, which might have aided in preserving the product. Total aerobic plate counts for beef smoked with *Acacia, Eucalyptus,* coconut shell and Neem wood are acceptable when compared to suggested range of 2.5×10^5- 1.0×10^8 cfu/g by Pearson (1996) as sited by Fakolade (2012).

Smoking which was done at high temperature contributed to the reduction in microbial load since most bacteria are not able to withstand the high temperatures encountered in the smoking process. When smoking is combined with curing, the shelf life of such products is increased and decreases the microbial load especially on the meat surface (Lawrie, 1990). This result can also be linked to the level of hygiene of the meat during processing as suggested by Uzeh *et al.,* (2006). Smoke constituents from *Acacia*, *Eucalyptus*, Neem wood and Coconut shell play important role in preserving the product against microbial spoilage.

5.8 Total Viable Counts, Coli-Form Counts of Suya

Microbial load of all suya samples (Table 4.8) fell within satisfactory region with reference to standard microbial load specification (Wilson *et al.,* 1991). Microbial load indicates extend of microbial contamination on the samples. Both pathogenic and non-pathogenic bacteria were observed, though within safe levels. The result from mean concentration of aerobic bacteria and coliform (56×10^4 - 2.15×10^6) were lower than values (2.9 - 9.8×10^6) reported by Ekanem (2000), on street vended meat (suya) product. Non-pathogenic microorganism when in amounts above recommended standards turn to pose some health hazards. The sources of these microbes in meat could be inherent micro-flora in normal tissues of animals, air, environment, or contamination due to unhygienic slaughtering, handling and processing conditions as suggested by Zattola, (1982).

This number could be further reduced by raising the number of Lactic Acid Bacteria (LAB) which are not as destructive as the non acid-tolerant species of microbes (Apata and Akinfemi, 2010). Similarly, higher LAB in *Suya* might be responsible for higher and better colour, flavour, texture and overall acceptability of *Suya.* The results obtained in this experiment are comparable to report by Venia *et al*. (2006) for dehydrated suya stored for 2 days post production. Alonge (1994) reported that application of salt, apart from eliminating non-salt tolerant bacterial specie by osmotic extraction of liquids through the cell walls; it also binds

water and makes it unavailable to microbes for growth. Similarly, results obtained from this study are in agreement with that reported by Igene and Mohammed (1993). According to the authors, the mean total count and coliform levels of 6.24×10^7 - 1.4×10^9 and 8.5×10^2 - 2.0×10^3/g were observed in suya products, emphasizing that Entero-bacteria are good meat contaminants.

5.9 Biochemical test results for Staphylococcus, salmonella, E-coli in smoked beef and suya samples

Bacteria isolated from the dried meat were *Salmonella* sp. (which was present in beef smoked using *Eucalyptus* and on suya made from *vastus lateralis),* and *E-Coli* (present in beef smoked using Cocunut shell and suya from *vastus lateralis* muscle) as shown in tables 4.9 - 4.12.

The low number and frequency of occurance of isolate can be linked to the level of hygiene of the raw meat and the various handling and processing methods, the low moisture content of samples and the bacteriostatic effect of the component smoke used which agreed with report by Uzeh *et al*. (2006). According to Frazier and Westhoff (1998) and Ekanem (2000) microbial growth on meat is higher prior to processing and lower after drying processes. This may explain the reduction in the types and number of micro-organisms that were isolated from the samples.

5.10 Moulds associated with Smoked beef samples

Fungi isolated include *Aspergillus niger, Aspergillus flavus, Rhizopus sp. Fusarium* sp. *Candida* sp. *and Penicillium* sp as shown in plates 1-6. Some of the isolated microbes are directly or indirectly involved in spoilage, although some may have been present as opportunistic contaminants which may not play any role in the spoilage of the dried meat samples. *Aspergillus* spp (isolated from all smoked samples, suya from *Bicep femoris, Semi membranosus* and *Vastus lateralis*) and *Rhizoppus* spp as shown in Plates 1 and 3, kept

growing on the substrate and spoilt the sample. The growth of xerophilic fungi on meat increases the water activity ($a_{w)}$ of the meat and after weeks of storage, it was noticed that the meat became softer thus allowing other organisms previously prevented by the low available water to now grow and contaminate the samples. Taniwaki *et al.*, (2001) reported that fungi are capable of growing at a very low moisture content or low water activity (a_w) of less than 0.83 a_w as well adapted to dry and partially dry food. *Aspergillus,* among the fungal isolates has been associated with disease conditions (Talaro, 2009). Results from this experiment are in agreement with findings revealing that *A. flavus* and *A. niger* are some of the fungal isolates of dried meat. Meat contamination by mold could also be as a result of contact of meat with soil (Rhodes, 1989). The potential health hazard of meat and meat products can be predicted on the basis of the normal water activity of the product with the minimal water activity to which indigenous microorganisms proliferate. Microbial spoilage, food poising and fermentation take place if the water activity of the substrate is favorable for the multiplication of the organism involved (Alonge, 1994). The effect of reduced water activity in a substrate creates a competition for water between dry solutes and organism nuclear enzymatic components as well as shrinkage effect. The organism must therefore cope with energy requirements of water replacement, water substitution, reproduction and maintenance or die (Labuza, 1990)

CHAPTER 6

6.0 Conclusions and Recommendations

Conclusion

- *Eucalyptus camaldutensis, Azadirachta indica* (Neem) and Coconut shell therefore are good sources of fuel wood for meat smoking as evidenced by the high crude protein and organoleptic scores of meat smoked with these new plant materials.
- The prime cuts, apart from resulting in Suya with high prices are not necessarily better than cuts from less choice parts of the carcass (*Semi membranosus, Semi-tendinosus, Biceps femoris, Rectus femoris* and *Vastus lateralis*) in terms of product yield and eating qualities.
- Microbial loads of smoked beef and *Suya* samples were within safe limit. Thus it is possible to produce suya with low microbial counts as long as smoking, seasoning and adequate hygienic conditions are maintained.

Recomendation

- It would be prudent to start using other trees species apart from *Acacia raddiana* and *I. doka* for meat smoking. The use of *Eucalyptus camaldutensis* should be encouraged as it will ease pressure off *A. Raddiana*. Tree products currently treated as waste like coconut shell can ease pressure off mangrove as a source of wood fuel in the south. The use of Neem tree in meat smoking as a possible deterrent to insect and mould infestation during storage can be investigated further for intrinsic properties of its smoke components.

REFERENCES

A.O.A.C (1990). *Official Methods of Analyses*. Association of Official Analytical Chemists, Washington D.C.15th Edition. 1: 931-946.

Abdullahi, I. O., Umoh V.J., Ameh, J. B. and Galadima, M. (2004) Hazards Associated with *Kilishi* Preparation in Zaria, Nigeria. *Nigerian Journal of Microbiology*.18 (1&2): 339 – 345.

Ajiboye, E., Alhassan, S., Adedayo, R., Majekodunmi, M., Kolawole, M., Olatunji, O. and Oladosu, T. (2011). Physicochemical properties and microorganisms isolated from dried meat obtained in Oja-Oba market in Ilorin, Nigeria (Pelagia Research Library) *Advances in Applied Science Research.* 2 (4):391-400.

Akande, G.R., Oladosu, H.O. and Tobor, J.G. (1996). Comparative Technical and Economic Appraisal of Fish Smoking: Two Traditional Ovens and a new improved Magbon-Alade Oven. FOA expert construction on Fish Technology in Africa. Kisumu, Kenya. *F.A.O. Fisheries Report* No. 574,

Alan, D.R.(2006).In vitro and In vivo studies of protein-protein interaction and macromolecular assembly. *Journal of Molecular Biology*. 35:496-507

Alonge, D.O. (1994). Smoke prservation of meats in Nigeria: Quality and Public health aspects, Ph. D. Thesis University of Ibadan, Ibadan.

Amanda, J.C. (2013). Processing and Preparation of Meat and Formation of Carcinogens. AICR'S Annual Research Conference on Food, Nutrition, Physical Activity and Cancer. November 7-8: 2913

Anjaneyulu, A.S., Thomas, R. and Kondaiah, R. (2007). Technologies for value added buffalo meat products-A Review. *American Journal of food Technology*. 2(3):104-114. http://dx.doi.org/10.3923/ajft.2007.104.114

Apata, E.S. and Akinfemi, A. (2010). Effect of different cooking methods on the proximate composition and eating quality of rabbit meat. *International Journal of Applied Agriculture* 6 (1 & 2):31-35.

Apata, E.S., Kuku, I.A., Apata, O.C. and Adeyemi K.O. (2012). Evaluation of suya, an Intermediate Moisture Meat Product in Ogun State, Nigeria. *Journal of Food Research*. 2 (1): 87-91

Arnim, F. and Yetti, M. (2012). Effect of liquid smoke utilization as preservative for meatballs quality. *Pakistan Journal of Nutrition*. 11(11): 1078-1080

Battacharyya, N., Chutia, M. and Sarma, S. (2007). Neem (*Azadirachta indica* A. *Juss*.), A Potent Biopesticide and Medicinal Plant. A Review. *Journal of Plant Sciences*, 2(3): 251-259.

Bendall, J.R. (1973). In: Bourne G.H. (Ed). *Structure and function of muscle*, Academic Press, New York, 2nd Edition. 2(2): 243-309.

Benjakul, S. and Aroonrueg, N. (1999), Effect of smoke sources on quality and storarage ability of catfish fillet (*Clarias Macrocephatus Gunther*). *Journal of food quality*. 22:213-224.

Beuchat, L. (1981) Microbial stability as affected by water activity. Cereal Foods World 26: 345-351.

Beuchat, L.R. and Toledo, R. T., (1994). Behaviour of *Byssochlamys nivea ascospores* in fruit syrups. *Trans. Br. of Mycology Society*. 68:65-71

Beuchat, L.T. and Jones, W.K. (1995). Effect of Food Preservation and Antioxidant on colony formation by heated conidia of aspergillus flavus. *Acta Alimentaria* 7: 373-384.

Biesalski, H. K. and Nohr, D. (2009). *The Nutritional Quality of Meat*. In: Kerry J.P. and Ledward D. (Eds). Improving The Sensory and Nutritional Quality of Fresh Meat, 1st Edition. Cambridge: Woodhead Publishing Ltd, England.1-148

Boles, J. A., Rathgether, B.M. and Shand, P.J. (2000).*Staphylococcus* in Salted Meat Product. *Journal of Meat Science.* 55**:** 22-231.

Cardinal, M., Corne, T.J., Serot, T. and Baron, R. (2006). Effects of Smoking Process on Odour Characteristics of Smoked Herring (*Clupea harengus*) and Relationships with Phenolic Compound Content. *Journal of Food Chemistry*. 96: 137-146.

Council for Agriculture Science and Technology, (CAST) (1997).”Contributions of animal products to Healthful Diets” CAST Task Force Report.

Cross, H.R., Savell, J.W. and Francis, J.J. (1996). National Consumer Retail Beef Study. In *Proceedings 38th Annual Reciprocal Meat Conference* 39: 112-114.

Dallyn, H. and Everton, J.R. (1969). Genus Xeromyces. https://books.google.nl/books?id=-

Dallyn, H. and Everton, J.R. (1997). Observations on the Sporicidal Action of Vegetable Oils Used in Fish Canning. *Journal of Applied Bacteriology*. 33: 603-608.

Duckworth, D. and Ormerod, M. B. (1997). Pupils’ Attitudes to Science. Slough, England: NFER.

Duncan, P.B. (1995). New Multiple Range and Multiple F-tests. Biometric 2(2):1-42.

Egbunike, G. N. and Okunbanjo, A. O. (2000). Effects of Processing upon the Quality of Nigerian meat products. *Livestock Production Science.* 59:155-163.

Ekanem, E.O. (2000). Application of Hazard Analysis Critical Point System to Quality Evaluation of Some Street Meat Products in Nigeria. *Asian-Austrailian Journal of Animal Science*. 13:325-327

Fabech, B. and Larsen, J.C, (1992). Smoking of food. A survey of method used in Denmark (in Danish). Publication no 135: 1-57.

Fakolade, P.O. and Omojola, A.B. (2008). Proximate Composition, pH and Microbiological Evaluation of kundi (Dried Meat) Product from Beef and Camel Meat. Tropeng University of Hohenheim, October 6-9. *Conference on International Research on Food Security, Natural Resource Management and Rural Development*.

Fakolade, P.O., (2012). Quality Characteristics and Microbiology Status in Kundi, an Intermediate Moisture Meat (IMM) Product. *Journal of Agricultural Science and Technology*. 2(7b):841-847

FAO (1995). Development and Promotion of Value Added Meat Products. *Project Document*.

FAO/WHO (2003). Assuring Food Safety and Quality: Guidelines for Strengthening National Food Control Systems. *Food and Nutrition.* Paper No. 76.

FAO/WHO (2003). The State of Food Insecurity in the World. Expert Consultation on Deit, Nutrition and the Prevention of Chronic Diseases. *Project Document*

Fernández, O.A. and Busso C.A. (1999). Arid and Semiarid Rangelands: Two Thirds of Argentina. In: Arnalds Ó, Archer S (Eds) *Proceedings of International Workshop of*

Rangeland Desertification Agricultural Research Institute. Reykjavic, Iceland. Rala Report Nº 200. : 41- 60.

Forrest, J.C., Aberle, E.D., Gerrard, D.E., Mills, W.E., Hedrick, H.B., Judge, M.D. and Merkel, R. A. (2001). *The Principles of Meat Science*. Kendall/Hunt Publishing Company: U.S. 4th Edition.

Frazier, W. C. and Westhoff, D. C. (1998)**.** *Food Microbiology*. International edition. Mc Graw Hill, Singaporepp 4th Edition. : 440-441.

Fritz, W. and Soos, K. (1991). Smoked Food and Cancer. *Bibl. Nutr. Dieta.* Pubmed-NCBI 29:57-64

Guillen, M.D. and Ibargoitia, M.L. (1999). Influence of the moisture content on the component of liquid smoke produced in the pyrolysis process of *Fagus sylvatica* L. wood. *Journal of Agriculture and Chemistry*. 47: 4126-4136.

Haman, D.O. (1977). Hemorrhagic cystitis and balantitis associated with verotoxin-producing *Escherichia coli* O157:H7. Lancetii: 150. *Microbiology of Meat Food Technology* 23 (6):66-71.

Harrigan, W.F. (2000). *Detection and Enumeration of Pathogenic and Toxigenic Organism - Pathogenic Escherichia Coli*. In: Harrigan, W.F. Laboratory Methods in Food Microbiology .3rd Ed. Academic Press, London.186-189.

Hedrick, H.B., Aberle, E.D., Forest J.C., Judge M.D. and Merkel R.A. (1994). Palatability and Cookery of Meat. *Principles of Meat Science* (3rd Ed) Kendal/hunt publishing Company, Dubuque. : 113-171.

Heymann, H.J., Hedndrick, H.B., Karrasch, M.A, Eggeman, M.K. and Ellersieck, M.R. (1990). Sensory and Chemical Characteristics of Fresh Pork Roasts Cooked to Different Centre Temperatures. *Journal of Food Science* 55:613-617

Hui Y. H. and Robert S.I. (2001). *Meat Science and Applications*. New York: Marcel Dekker. ISBN 978-0-8247-0548-0.

Hunt, M.C., Sorheim, O. and Sindle, E. (1999). Colour and Heat Denaturation of Myoglobin Forms in Ground Beef. *Journal of Food Science*. 64(5):847-851

Igene, J.O. and Mohammed, I.D. (1993). Consumer Preferences and Atitude to *Suya*: An Indigenous Meat Product. *Annals of Borno.* 1:169-178.

Ikeme, A. I. (1990). *Meat Science and Technology*: A Comprehensive Approach. African-Fep. Publishers Limited. :12-16

Inneke, H. (2011). Food Material Science. https://sintak.unika.ac.id/staff/blog/uploaded/5812002253/files/pengetahuan_bahan_2011/eggs

Inyang, C.U., Igyor, M.A. and Uma, E.N. (2005). Bacterial Quality of a Smoked Meat product (*Suya*). *Nigerian Food Journal.* 23: 239-242.

ISO (2001). Microbial – Horizontal Method for the Detection of *Escherichia coli* O157:H7 First Edition. International Organization for Standardization, Geneve, Switzerland.

Ketkar, C.M. (1986). Utilization of neem (Azadirachta indica Juss.) and its by-product. Hyderabad, India: Khadi and Village Industries Commission, Directorate of Non-Edible Oils and Soap Industry, *Final Technical report*. 279

Khandaghi, J., Vadood, R. and Abolfazi, B. (2010). Isolation of *Escherichia coli* O157:H7 from manure fertilized farms and raw vegetables grown on it, in Tabriz city in Iran. *African Journal of Microbiology Research,* 4 (9):891-895.

Kjällstrand, J. and Petersson, G. (2001). Phenolic antioxidants in alder smoke during industrial meat curing. *Journal of Food chemistry*. 74: 85-89

Koohmaraie, M. (1995). The Biological Basis of Meat Tenderness and Potential Genetic Approaches for its Control and Prediction. *Proceeding Rec. Meat Conference.* 48:69-75.

Koohmaraie, M. and Geesink, G.H. (2006). Contribution of Postmortem Muscle Biochemistry to the Delivery of Consistent Meat Quality with Particular Focus on the Calpain System. *Journal of Meat Science.* 74:34-43.

Koohmaraie, M., Kent M.P., Shackelford, S.D., Veiseth, E. and Wheeler, T.L. (2002). Meat Tenderness and Muscle Growth: is there any Relationship? *Journal of Meat Science.* 62: 345-352

Kropf, D. H., Stroda, S. L., Seyfert, M., Hunt, M. C., and Mancini, R. A. (2004). Internal Premature Browning in Cooked Steaks from Enhanced Beef Round Muscles Packaged in High-oxygen and Ultra-low oxygen Modified Atmospheres. *Journal of Food Science*. 69:142–146.

Labuza, T.P. (1990) Storage Stability and Improvement of Intermedidiate Moisture Foods. Final Report, Contract No. NAS 9-10658, NASA, *Food Nutrition office, Houston, TX*.

Laird, D.A., (2006). *Food Material Science*. Inneka Hantoro. www.ars.usda.gov/SPD2US../2006.pdf

Lawrie R.A. (1990). *Meat Science* (4th Ed.). Pergamon Press, Oxford, United Kingdom. pp136

Lawrie, R.A. (2003). Health Libraries Review. *Health Information and Libraries Journal*. 1:1365-2532.

Lawrie, R.A. and Ledward D.A. (2006). *Lawrie's Meat Science* (7th Ed). Cambridge: Woodhead publishing limited. England and CRC Press Boca Raton, New York, Washington DC ISBN 978-84569-159-2.

Leistner, L. and Rodel, W. (1996). The Stability of Intermediate Moisture Foods with Respect to Micro Organisms. In; Intermediate Misture Food. London, *Elsevier Applied Science.* 120-137

LeJeune, J.T., Besser, T.E., Rice, D.H., Stilborn, R.P. and Hancock, D.D. (2004). Longitudinal Study of Faecal Shedding of *Escherichia coli* O157: H7 in Feedlot Cattle: Predominance and Persistence of Specific Clonal Types Despite Massive Cattle Population Turn Over. *Applied Environmental Microbiology*. 70:377-384.

Levine, M. (1994). *Escherichia coli* infections. In: Bacterial vaccines. Germanier R. (Ed) Hemorrhagic cystitis and balantitis associated with verotoxin-producing *E. coli* syndrome associated with a variant shiga-like cytotoxin of *Escherichiacoli* O111. *Pediatric Infectious Disease Journal.* 7:128-132.

MacFaddin, J.F., (2000). Biochemical tests for identification of medical bacteria, 3rd Ed. Lippincott Williams and Wilkins, Philadelphia, P.A.

Mahendraker, N.S., Khabade, V.S. and Dani, N.B. (1988). Studies on the effects of fattening on carcass characteristics and quality of meat from Bannu-lamb. *Journal of Food Science Technology* 25: 228-230.

Mark, G. and William, C (2001). Global Food Consumption and Impacts on Trade Patterns. *U.S. Department of Agriculture, Agriculture and Trade Report*. Diane publishing.WRS-011.

Martinez, O., Salmeron, J., Guillen, M.D. and Casas, C. (2007). Sensorial and physicochemical characteristics of salmon (salmo salar) treated by different smoking processes during storage. *Food Science and Technollogy International.* 13(6): 477-484.

Mboung, C.M.F. (1999). The Effect of Traditional African Method of Meat Processing on the Finished Product (Kilishi) following in-vitro enzymolysis in: Schlemer U. (ed), Bioavailablility 93, Nuritional, chemical and food processing implications of nutrient availability, proceedings part 2, 169-174.

McGee, H., (2004). *On food and Cooking* (revised edition). Scribner Publisher: 427-429

Miller, N. and Golding, N. S. (1989). The Gas Requirements of Molds. IV. A preliminary interprecarion of the growth rates of our common mold cultures on the basis of absorbed gases. *Journal of Diary Science*. 2(8): 717-750.

Moon, S.S. (2006). The Effect of Quality Grade and Muscle on Collagen Contents and Tenderness of Intramuscular Connective Tissue and Myofibrillar Protein for Hanwoo Beef *Asian-Australian Journal Animal Science*. 19(7):1059 – 1064

Myrvold, N. (2011). Modernist cusine. The cooking lab. : 143. ISBN 978-0-9827610-0-7

Neidhardt, C.F. (1995). *Escherichia coli* and *Salmonella tyhimurium* In: *Cellular and Molecular Biology*, vol. 2. American Society for Microbiology, Washington D.C., 1639-1640.

Obanu, Z.A. (1981). The applicability of Intermediate Moisture Food (IMF) technology for preservation of meat and fish in Nigeria. Paper presented at the National *Conference on 'From Food Deficiency to Food Sufficiency'* at the Rivers State University of Science and Technology. Port Harcourt, May 3-8.

Odote, P.M. (2006). Improved Traditional Fish Processing method by Smoking and Solar drying in Tana River and South area of Kenya. WIOMSA MARG 1 *Technical report.*

Oduor-Odote, P.M., Ohowa, B.O. and Obiero, M. (2008). Performance of Improved Smoking Kilns Introduced at the Tana-Delta Region of Kenya. Samaki News- *A Magazine of the Department of Fisheries, Kenya.*1: 23-28.

Odusote, K.A and Akinyanju, O.O (2003) Microbiological evaluation of Suya sold in Adoand Akure, South West Nigeria *Consumer Safety Bulletin*, 2: 20 – 24.

Offer, G. (1984). *Progress in the Biochemistry, Physiology and Structure of Meat*, in Proceedings of the 30th European Meeting of Meat Research Workers Bristol UK. 87.

Ogunbanwo, S.T., Sanni, A.I. and Onilude, A.A. (2004). Effect of Bacteriocinogenic *Lactobabillus spp*. On the shelf life of fufu, a traditional fermented cassava product. *World Journal Microbiol*ogy and *Biotechnology*.20: 57-63.

Okoli, I.C., Okorondu, U.V. and Opara, M.N. (2006). Environmental and Public Health Issues of Animal Food Products Delivery System in Imo State, Nigeria. *Online Journal of Health and Allied Sciences* 5(2).http://www.ojhas.org/issue18/2006-2-2.htm.

Okubanjo, O.A., Omojola, A.B., Ogunsola, O.O., Adewumi, M.K., Ajiboro, G, Alabi, G.F. and Babayemi, O.J. (2003). Meat Characteristic of Bunaji, Gudali and Keteku breeds of cattle. *Tropical Journal of Animal Production* 6: 185

Okubanjo, A.O. (1990). *Meat for the Nigerian Millions*. Faculty lecture series, Faculty of Agriculture and Forestry, University of Ibadan, Nigeria. No.3.

Omojola, A. B, Kassim, O.R., Adewumi, M.K., Ogunsola, O.O., Adeyemo, G.O. and Deshiyan, A.B. (2004). Evaluation of the effects of variation in ingredient composition on the eating qualities of suya. *African Journal of Livestock Extension.* 3: 28-32.

Omojola, A.B., Isah, O.A., Adewumi, M.K., Ogunsola, O.O. and Atta, S. (2003). Evaluation of Efect of Different Additives on Acceptability of Kilishi *Tropical Journal of Animal Science.* 6(2): 97-101

Omojola, A. B. (2008). Yield and Organoleptic Characteristics of Suya (an intermediate moisture meat) prepared from three different muscles of a matured bull. *African Journal of Biotechnology, Vol. 7*(13), 2254-2257.

Pearson, A.M. (2012). Biochemical changes in Beef Muscle Post-mortem and Effect of Ice Cooling. *Journal Food Science Agriculture.* 18: 314.

Pearson, D. (1996). Application of Chemical Methods for assessment of beef quality. *Journal of Food Science, Food Agric.* 19: 364-366.

Pfeiffer, K.D., Mckenna, D.R. and Baird, B.E. (2005). Biochemical and Physical Factors Affecting Discoloration Characteristics of 19 Bovin Muscles. *Journal of Meat science.* 70:665-670

Rajasekhar, N., Sasikala, C. and Ramana, C.V., (2000). Toxicity of N-containing Heterocyclic Aromatic Compounds and Their Utilization for Growth by a Few Purple Non sulfur Bacteria. *Bull Environmental Contaminant Toxicology*. 65:375-382.

Reddy, C.A. (2007). *Methods for General and Molecular Microbiology*, 3rd Ed. ASM Press, Washington D.C.

Reddy, M. (2007). Role of FtsEx in cell division of *Escherichia coli*: viability of ftsEx mutants is dependant on functional stuff or high osmotic strength. *Journal of bacteriology*. 189(1): 98-108

Rendle, R.C. and Keeley G.(1998). Chemistry in the Meat Industry. A Review. *Journal of Animal Product.* http://nzic.org.nz/ChemProcesses/animal/5A.pdf

Rhodes, M. (1989). *Fungi Associated with Meat. Food mycology*. Boston. G.K. hall. 102-105

Rockland L.B. and Beuchat L.R. (1997). Water Activity: Theory and Application to Food. Marcel Dekker, New York. 203-204

Ronald, B. and Fereidoon, S. (2004). Nitrite curing of meat: the N-Nitrosamine problems and Nirtite alternative

Ryan, K. and Ray, C. (2004). *Sheris Medical Microbiology*. McGraw Hill Society for Microbiolology. 407-417

SAS. (1999). Statistical Analysis System, Computer Software, Version 9: Statistics SAS Institute Inc. Cary, NC 27513, NC 27513, USA.

SAS. (2002). *Statistical Analysis System.* SAS Stat. Version 9 SAS Institute Inc. Garry, NC, USA.

Schurgers, L.J. and Vermeer, C. (2000). Determination of Phlloquinone and Menaquines in food. Effect of food matrix on circulating vitamin K concentration. *Haemostasis* 30(6): 298-307

Seideman S.C. and Cross C. (1995). Sex, Age and Breed Related changes in Bovine Testosterone and Intramuscular Collagen. *Journal of Animal science.* 55: 826

Scott, W.J. (1977). Water relations of food spoilage microorganisms. *Advances in Food Research.* 7:83-127.

Simko, P. (2005). Factors Affecting Elimination of Polycyclic Aromatic Hydrocarbons from Smoked Meat Foods and Liquid Smoke Flavorings. A Review. *Molecular. Nutrition Food Research*. 49: 637-647

Soniran, O.G., and Okubanjo A.O. (2002) Physico-chemical and sensory characteristics of pork loin roast cooked to three internal temperatures. Nigerian Journal of Animal Production 29 (1): 138-141

Sorheim, O., Nissen H., and Nesbakken T. (1999). The storage life of beef and pork packaged in an atmosphere with low carbon monoxide and high carbon dioxide.*Journal of Meat Science*. 52(2): 157–164.

Suzuki, A, Koima N. and Ikeuchi Y. (1997). Carcass composition and meat quality of Chinese purebred and European X Chinese crossbred pigs. *Journal of Meat* Science. 29: 31-41.

Swatland, H.J. (1994). Update on the connective tissue probe. Beef Research Update, OAC Publication 0394:27-29

Talaro, K.P. (2009). Foundations in Microbiology, seventh edition. The McGraw-Hill Companies Inc.

Taniwaki, M. H., Hocking, A. D., Pitt, J. I. and Fleet, G. H. (2001) Growth of fungi and mycotoxin production of cheese under modified atmospheres. *International Journal ofFood Microbiology* 68**:**125–133.

Todar, K. (2002). Pathogenic *Escherichia coli* (internet). University of Wisconsin-Madison, Department of Bacteriology. Available from: http://textbookofbacteriology.net/e.coli.html

Tornberg, E. (2005) Effect of heat on meat protein- implications on structure and quality of products. *Journal of Meat Science* 70: 493-508.

Torngren M. A. (2003): Effect of packing method on colour and eating quality of beef Loin Steaks. *In 49th Proceedings of International Congress of Meat Science and Technology*. Campinas, Brazil, 495-496.

Toth, L. and Potthast, K. (1984). Chemical Aspects of Smoking Meat and Meat Products. *Advances in Food Research.* 29:87-158.

Troller, J.A. and Christain, J.H.B., (1990). *Water activity in food.* Academic press, inc., New York.

USDA-FSIS, U.S. Department of Agriculture–Food Safety and Inspection Service. 2004. Compliance guideline for meat and poultry jerky. USDA-FSIS, Washington D.C., http://www.fsis.usda.gove/OPPDE/nis/outreach/models/Jerkyguidelines.htm. .

USDA-FSIS (1999) Bacon and Food safety. www.fsis.usda.gov/wps/portal/fsis/topics/food-safety-education/get-answers/food-safety-fact-sheets/meat-preparation/bacon-and-food-safety/ict_index

Uzeh, R.E, Ohenhem, R.E. and Adeniji, O.O (2006): Bacteriological Contamination of Tisre-Suya, a Nigerian meat product. *Pakistan Journal of Nutrition*. 5(5): 458-460.

Torres, E.A.F.S., Shimokomaki, M., Franco, B.D.G.M. and Landgrant, M. (1994). Parameter determining the quality of charqui an Intermediate Moisture Meat Product. *Journal of Meat Science*. 38:229-234

Venia, F., Joana, B., Sandra, V., Ana, M., Fatima, S., Maria, J.M., Tim, H., Paul, G. and Paula, T. (2006). Chemical and Microbiological Characterisation of *Alheria*; A typical Portuguese fermented sausage with particular reference to factor relating to food safety. *Meat Science Journal*. 73(4): 570-575.

Wagner, A.B. (2010). Bacterial poisoning. http://generalhealthtopics.com/bacterial-food-poisoning-330.html. Retrieved on 02/08/2010.

Waxman, J.S., Gamble, C., Chotai, D. and Odontiadis, M. (2007). Prohibitin, a protein downregulated by androgens, represses androgen receptor activity, *Oncogene*, ISSN:0950-9232, 26:1757-1768

Wikipedia (2013a). Smoking House. http://en.m.wikipedia.org/wiki/smoking_cooking

Wikipedia (2013b). Beef cut. http://en.m.wikipedia.org/wiki/cut_of_beef

Wilson, D. and Sperber, D. (1991) On Grice's theory of conversation. In P. Werth (ed.) *Conversation and Discourse.* London: Croom Helm. 155-78. Reprinted in Kasher. 1(5):347-68.

Womack, R.M. (2010). *The anthropology of health and healing.* Rowman and littlefield. P243. ISBN 0759110441.

Zattola, E.A. (1982). *Introduction to meat Microbiology,* American Meat Institute, Chicago, USA. 245-253