DETERMINATION OF PROXIMATE COMPOSITION, MINERAL ELEMENTS, HEAVY METAL LEVELS AND MICROBIAL QUALITY OF *KILISHI* FROM SELECTED AREAS IN KADUNA STATE, NIGERIA

BY

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DECLARATION

I declare that the work in this dissertation entitled **"DETERMINATION OF PROXIMATE COMPOSITION, MINERAL ELEMENTS, HEAVY METAL LEVELS AND MICROBIAL QUALITY OF *KILISHI* FROM SELECTED AREAS IN KADUNA STATE, NIGERIA"** has been carried out by me in the Department of Chemistry, under the supervision of Prof. E. B. Agbaji and Prof. V. O. Ajibola. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previosly presented for another degree or diploma at this or any institution.

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Signature Date

CERTIFICATION

This dissertation titled **"DETERMINATION OF PROXIMATE COMPOSITION, MINERAL ELEMENTS, HEAVY METAL LEVELS AND MICROBIAL QUALITY OF *KILISHI* FROM SELECTED AREAS IN KADUNA STATE, NIGERIA"** by Thomas Saleh SHEYIN meets the regulations governing the award of the degree of Master of Science (M.Sc.) in Analytical Chemistry of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

I dedicate this work to my late father Sheyin Bahuyak of blessed memory and to my dear aged mother Victoria Sheyin who nurtured me in all spheres of life.

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I am most grateful to Almighty God for making me who I am and for His guidance, protection, good health and strength given to me throughout this period. Praise Be to Him for His Mercy.

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ABSTRACT

Analysis of proximate composition, mineral composition, heavy metals concentration, microbiological counts and identification of microbial contaminants were carried out in beef and *Kilishi* samples that were produced and collected from six production spots in Kaduna state, Nigeria viz: Sabon Gari, Tudun Wada, Zaria city, Malali, Anguwan Sarki and Kasuwa. Also, *Kilishi* that was prepared in the Laboratory that served as control was analyzed. Results of proximate composition showed that *Kilishi* is richer in nutrients. It showed that the profile proximate parameters in the study samples is protein > crude fat > ash > moisture > carbohydrate > crude fibre. The highest protein content found was in dried beef. From the study samples, Kasuwa *Kilishi* had the highest protein content (64.28%), Malali *Kilishi* had the highest moisture and fat contents (9.25 and 22.42% respectively), while Anguwan Sarki *Kilishi* had the highest ash content (9.42%). Also, Tudun Wada *Kilishi* had the highest crude fibre content (3.42%), Zaria City *Kilishi* had the highest carbohydrate content (8.32%), while the Laboratory *Kilishi* that served as control recorded the lowest percent moisture (7.22%) and had lower values of protein and carbohydrate. Results of mineral composition showed that *Kilishi* contained the highest contents of Na, P and K while the concentrations of Ca and Mg found were highest in dried beef. Kasuwa *Kilishi* had the highest contents of Na (8281.00) and Mg (474.55) compared to other *Kilishi* samples. The highest values of P (8915.00) and K (9610.00) obtained were from Malali *Kilishi*, Tudun Wada *Kilishi* had the highest Ca content (459.55), while the Laboratory *Kilishi* had the lowest mineral contents of Ca, Mg and P. The results revealed that *Kilishi* contained the highest concentrations of the heavy metals. The concentrations ($mgkg^{-1}$) of Fe (142.18 - 192.80), Zn (80.09 - 135.43), Pb (1.83 - 13.75) and Ni (2.08 - 19.88) in this study exceeded their threshold effects levels

(80.00, 50.00, 0.50 and 0.50 $mgkg^{-1}$/day respectively). From the study samples, Co and Mn contents (0.00 - 0.68 and 10.95 - 19.50 respectively) exceeded their threshold effects levels (0.16 and 11.00 $mgkg^{-1}$/day respectively); except in *Kilishi* samples from Sabon Gari and Zaria City were Co was below detection limits. However, Cd and Cr were below detection limits in all the samples, while Cu contents (2.84 - 7.58) in the entire sample were below the threshold effects levels 8.0 $mgkg^{-1}$/day). The Laboratory *Kilishi* had Co and Mn contents that were below threshold effects levels. Tudun Wada *Kilishi* had the highest Fe value, Sabon Gari *Kilishi* had the highest Zn value, Zaria City *Kilishi* had the highest Pb value, while Sabon Gari *Kilishi* had the highest Ni value. Microbiologically (cfu/g), *Staphylococcus* and fungi counts in all the samples (1.56×10^5 - 3.63×10^7 and 1.01×10^4 - 2.02×10^5 respectively) were above WFP recommended limits 10^6 and 10^2 respectively. Coliforms counts (0.00 - 9.61×10^6) were above WFP threshold limits in some of the study samples. However, the Mesophillic aerobic counts (0.00 - 9.03×10^5) were below the threshold effects limits of 10^6 with Tudun Wada *Kilishi* having the highest value. Microbial contaminants of *Staphylococcus aureus*, *Bacillus* spp., *Salmonella, Klebsiella, Escherichia coli, Aspergillus, Penicillium* and *Geotricum candidum* were isolated in some samples with *Staphylococcus aureus* predominantly detected in all the samples. The Laboratory *Kilishi* had Mesophillic counts below detection limits, Coliform counts below the threshold effect value and *Bacillus, Salmonella, E. coli, Aspergillus* and *Penicillium* species not detected.

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LIST OF ACRONYMS AND ABBREVIATIONS

APC	=	Aerobic plate count
ATP	=	Adenosine triphosphate
cfu	=	Colony forming unit
EC	=	European Communities
EMBA	=	Eosine methylene blue agar
FAO	=	Food and Agricultural Organization
LCB	=	Lactophenol cotton blue
MSA	=	Mannitol salt agar
PDA	=	Patato dextrose agar
RDA	=	Recommended Dietary Allowance
SDA	=	Sabourad dextrose agar
WFP	=	World Food Programme

CHAPTER ONE

I.0 INTRODUCTION

I.1 Background

Meat and meat products are important for human diet because they provide the needed proteins, minerals and trace elements. Meat and meat products are extremely perishable mainly because preservation app.liances are hard to come by and where they are available, erratic power supply in the country is a main problem. Many methods of preserving meat have been used throughout history such as the use of high temperature (e.g. canning), low temperature (e.g. chilling), freezing and pasteurization, drying (e.g. hot air drying, wind and sun drying), smoking, use of radiation and the use of chemical preservatives (Alonge and Hiko, 1981).

As a means of preserving meat, the early Fulani and Hausa herdsmen of Northern Nigeria and the Sahelian Africa have introduced *Kilishi* (Alonge and Hiko, 1981). The use of seasonings such as salt, spices, herbs and fermented sauces had been employed to enhance the acceptability of traditionally processed meat product like *Kilishi*. Study has revealed that *Kilishi* has a shelf life of 12 months at room temperature (Igene *et al.*, 1990).

The pollution of the environment with heavy metals has become a worldwide problem and of scientific concern because the metals are indigestible and most of them have toxic effects, which constitute a potential danger to humanity (Khan *et al.*, 1996). Heavy metal pollution is a serious threat to the high demand of *Kilishi* because of the toxic effect which they impact in the *Kilishi*, bioaccumulation and biomagnifications in

the food chain (Demirezen and Uruc, 2006). In addition, the microbiological pollutants acquired through the food chain of *Kilishi* products poses serious danger of diseases to humans. Toxicity is realized when these heavy metal levels are higher than the recommended limit which varies for individual elements in meat and meat products.

There are over ten (10) heavy metals including cobalt (Co), lead (Pb), mercury (Hg), arsenic (As), thallium (Tl), nickel (Ni), manganese (Mn), zinc (Zn), cobalt (Co), cadmium (Cd) and chromium (Cr) that have particular significance in ecotoxicology, since they are highly persistent (Storelli *et al.*,2005). The levels of metals such as Mn, Zn, Co and Cr are toxic beyond a certain limit, whereas Pb, Ni and Cd are toxic even in trace amounts (Bury *et al.*, 2003; Fernandes *et al.*, 2008). Therefore, this study determined the mineral contents, heavy metals levels and microbiological contamination in *Kilishi* from different production spots in Kaduna state, Nigeria to establish if the levels were within the recommended limits by World Health Organization. The quality of the meat used for the production of the *Kilishi* samples was also investigated.

1.2 Statement of the Problem

The traditional method of *Kilishi* preparation in Kaduna State, Nigeria has the risk of exposure to heavy metal pollutants from the environment through vehicle emission, dirty slaughter places and dust. The producers of these meat products prepare it in open markets and even on roadsides. This may result in contamination and thus bioaccumulation of heavy metals in people who consume these products. The analysis of heavy metals that include iron, copper, zinc, cobalt, manganese, lead, cadmium, chromium and nickel, is therefore justified to provide precautions towards the use of the

Kilishi products, as well as provide a basis to improve on its quality. This study will also serve to sensitize government authorities such as National Environmental Management Authority (NEMA) towards identifying sources of heavy metals pollution in the local places of *Kilishi* production and for systemic toxic heavy metals and microbiological control before and after the production of *Kilishi*.

1.3 Justification

With the increased awareness and consumption of the *Kilishi*, this study was conducted in view of the scarcity of information of the nutritive quality and levels of heavy metal contamination in *Kilishi* popularly consumed in selected areas in Kaduna state of Nigeria.

1.4 Aim and Objectives

The aim of this research is to determine the proximate composition, mineral content, heavy metals composition and microbial contaminations of *Kilishi* samples, differently prepared from the same beef source, at some selected *Kilishi* selling spots in Kaduna State of Nigeria. This aim would be achieved through the following objectives:

i. To prepare *Kilishi* by commercial producers, from the same beef source, at six-selected *Kilishi* selling spots in Kaduna State of Nigeria viz: Kasuwan Mata-Sabon Gari in Zaria, Yan Awaki-Tudun Wada in Zaria, Durumi Maigarke-Zaria City in Zaria, Raba Road-Malali in Kaduna, Sultan Bello Road-Anguwan Sarki in Kaduna and Kasuwa-Jos Road in Kaduna.
ii. To prepare *Kilishi* and raw beef in the oven as control samples, from the same beef source, in the laboratory.

iii. To determine the proximate composition (moisture content, ash content, fat content, protein content, fibre content and carbohydrate) in the prepared samples.

iv. To determine the mineral content (Na, Ca, Mg, P and K) in the samples.

v. To determine selected heavy metals (Fe, Cu, Zn, Co, Mn, Pb, Cd, Cr and Ni) level in the samples.

vi. To determine the microbial quality (Mesophillic aerobic plate count, *Staphylococcus* count, Coliform*s* count and fungal count) and identify some selected bacteria and fungi species in the samples.

vii. To compare the results obtained of *Kilishi* prepared by Commercial producers from their respective production spots with values of the control samples prepared from the laboratory.

viii. To compare the results obtained with WHO and FAO standard limits for meat and meat products.

1.5 Hypothesis

For this research, it is hypothesized that the levels of heavy metals concentrations and microbiological contamination in *Kilishi* meat products produced in six selected spots in Kaduna State, Nigeria do not differ significantly from the recommended limits specified by the International standards of World Health Organization (WHO) and Food and Agricultural Organization (FAO).

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Meat and its Composition

Meat is generally define as the flesh from domesticated animals and generally includes the skeletal tissues or flesh of cattle, sheep and other animals (Sulzbacher and Gaddis, 1977; Potter, 1980). The Food Safety and Inspection Service (2001) defined meat as "All parts of an animal that are intended for, or have been judged as safe and suitable for human consumption." Thus, meat is the edible portion of animals, which is suitable for use as food and consists of lean and fatty tissues. In many developing countries, especially Nigeria meat is widely consumed either cooked or processed into other forms to avoid associated spoilage (Olaoye *et al.*, 2010).

Chemically meat is composed of four major components including water, protein, lipid, carbohydrate and many other minor components such as vitamins, enzymes, pigments and flavour compounds (Lamber *et al.*, 1991). According to Beilken *et al.* (2007), every specie of meat has its chemical characteristics. Lawrie (1991) reported that chemical composition of meat comprises 56-72% water, 15-22% protein, 5-34% fat, and 3.5% soluble non-protein substances (including carbohydrates, organic salt, dissolved nitrogen substances, minerals and vitamins). Aberle *et al.* (2001) reported that meat contains 70% water, 19% protein, 5% fat, 3.5% non-protein substances, and minerals and other materials 2.5%. However, Rendle and Keeley (2000) reported that each species has different chemical characteristics. In their report, the chemical composition of meat changes after slaughtered due to the stiffness of the dead muscles, a process known as 'rigor mortis.' Therefore, at this rigor mortis, the muscles glycogen would be converted

into lactic acid that caused a falled in pH making the meat to lose some of its water-binding capacity a desirable feature that slows down the growth of microorganisms and enhances flavour, juiciness and colour of the meat to give an attractive saleable product.

2.1.1 Water

The moisture content of meats depend on several factors and varies considerably with age of the animal, species, fat contents and the particular tissue under consideration (Greenfield *et al.*, 1987). Rendle and Keeley (2000) reported the water content in lean meat, beef and lamb of 75, 73 and 71% respectively.

2.1.2 Protein

Muscle protein is divided into 3 groups based on their solubility properties; firstly, sarcoplasmic protein (30%), myofibrillar protein (55%) and stromal protein or muscle tissue (15%) (Droulez *et al.*, 2002). Williams (2007) reported that the sarcoplasmic proteins are mostly glycolytic enzymes (glucosidase, diastase, pepsin, trypsin, among others) and are involved in conversion of stored energy into muscles power. He also reported that the myofibrillar protein such as myosin and actin are responsible for the muscle's overall structure. Chan *et al.* (1995) reported that stromal protein such as collagen and elastin affects the meat quality by lowering the tenderness of the meat, which depends on its number and degree of its cross-linking to other stromal proteins. It also influences the meat emulsion capacity, due to its non-solubility in water and lowers water-binding capacity of the meat because of its low content of hydrophilic and charged amino acids.

According to Williams (2007), raw red muscle meat contained around 20 to 25 g protein per 100g, while cooked red meat contained 28 to 36 g per 100g due to the

decreased in water content that makes its nutrients become more concentrated during cooking. Protein from meat provides all essential amino acids: lysine, threonine, methionine, phenylalanine, tryptophan, leucine, isoleucine, valine, and has no limiting amino acids. It performs a wide range of functions such as provision of energy (5.65Kcal/g) that contribute 15 - 20% of the total calories in the body for maintenance (Pushparajan *et al.*, 2012).

2.1.3 Fat

Fat in meat is generally in triglycerides form that significantly determines the meat tenderness and roughness. It is an important energy source because the amount of energy produced can be double from that generated by proteins and carbohydrates. According to Lawrie (1991), other important substances such as vitamins: A, D, E and K are dissolved in fat. Kris (2013) reported that fat contains nine calories per gram, while protein contains four calories per gram. He reported that fatty meats tend to be higher in fat-soluble vitamins such as vitamins A, D, E and K_2 than in lean meats. Rendle and Keeley (2000) reported fat content in lean, beef and lamb meats of 3.0, 3.9 and 7.4% respectively.

2.1.4 Organic substances (soluble non-protein)

These substances fall into two categories: nitrogen-based substances such as free amino acids and vitamins, and carbohydrates such as lactic acid and glucose (Kris, 2013). According to Kris (2013), meat is a good source of essential amino acids, minerals and vitamins, and a good source of energy (on average, beef yields 510 kJ and lamb yields 630 kJ per 100 g) and all the essential amino acids required for life are present and highly

bioavailable in raw muscle tissue. Most importantly, meat is a dietary source of both vitamins B_1 (thiamin) and B_2 (riboflavin).

2.1.5 Inorganic substances

The importance of meat as an essential source of some micronutrients is because it is either their only source, or have a higher bioavailability (Kris, 2013). The major minerals in meat consist of phosphorous and potassium together with small amounts of sodium, magnesium, calcium, zinc, and other trace metals (Rendle and Keeley, 2000). The Author also reported that the other soluble minerals (amino acids, vitamins and carbohydrates) and the mineral contents (mostly K, P, and amounts of Na, Mg, Ca and Zn) in lean meat, beef and lamb meat are 4.0, 1.1 and 1.0% respectively.

2.2 Beef

"Beef" is said to be meat from full-grown cattle of about two years old, whereas meat of young cattle that are less than three or four months old and are milk fed calves is known as "veal" (Sulzbacher and Gaddis, 1977). Usually, beef is red colour and is sometimes called 'red meat' because it contains more myoglobin (1-3 mgg^{-1} tissue in calves and 16 - 20 mgg^{-1} tissue in old beef cattle) and is universally acceptable compare to veal and other meats (Lawrie, 1981). Although producers of "natural" and "organic" beef products use production methods that differ from conventional ones, these types of beef have not been shown to differ from other beef (grain-fed and grass-fed beef) in terms of nutrition or safety (Migdal *et al.*, 2009).

Lawrie (1985) analyzed mineral contents of beefsteak raw, grilled, and reported that potassium (K) was highest (334.0 and 368.0 $mg100g^{-1}$ respectively) followed by

phosphorus (P) (278.0 and 303.0 $mg100g^{-1}$ respectively). In this study, sodium (Na) in beefsteak raw and grilled was found to be 69.0 and 67.0 $mg100g^{-1}$ respectively, magnesium (Mg) found was 24.5 and 25.2 $mg100g^{-1}$ respectively while calcium (Ca) was 5.9 and 9.2 $mg100g^{-1}$ respectively. He concluded that the heat source and the metal used for the meat grilled might contribute to its increased in mineral contents. Okubanjo (1997) in his study concluded that beef contains 18-23% of proteins. In his study, he reported that beef is rich in mineral compounds especially phosphorus and iron, involves more iron of about 35% of its daily dietary level intake than in other meats and a main source of vitamins B_{12}, B_1 and B_6. According to Migdal *et al.* (2009), beef is parasite-free meat, used as culinary meat and rich in mineral compounds especially phosphorous, zinc and iron.

2.3 *Kilishi*

Kilishi is a sun dried traditional meat product made principally from beef. An intermediate moisture meat that has a suitable concentration of dissolved solids that bind its moisture sufficiently to inhibit the growth of spoilage organism (Igene *et al.*, 1990). *Kilishi* is a ready-to-eat convenience meat products possessing excellent shelf stability at room temperature. Handling and marketing of *Kilishi* products should be made convenient for both consumers and retailers alike (Igene *et al.*, 1990; Olusola, 2006). Igene (1988) reported that *Kilishi* contained about 46% meat and 54% non-meat ingredients. Igene *et al.* (1993) reported that the finished *Kilishi* product contained 50% protein, 7.5% moisture, 18% lipid, 9.8% fibre and 14.7% ash.

The popular sun-dried beef *Kilishi* has gained wide acceptance not only in Nigeria but also across the sub-Sahara region and beyond. Borno, Kano, Sokoto, Kaduna and Bauchi in Northern Nigeria are the most *Kilishi* producing states. *Kilishi* production is possible in the states because of the dry winds, high temperatures and low humidity that usually characterize the North and Far North regions. In addition, the population of the cattle of more than 70% of the animals in these regions and the high demand of Kilishi by the inhabitants, "Hausa and Fulani" of these regions encouraged the production of Kilishi in this Northern part of Nigeria (Alaku and Igene, 1983). Production of *Kilishi* in the southern states of Nigeria is limited to the longer rainy season that comes with high atmospheric moisture. However, the Northern Nigeria dry season might cause the air to be polluted with dust particles, carrying adhering microorganisms, which can cause important human diseases.

Kilishi is usually prepared by partially drying thin sheets of quality meat in the sun, followed by marinating the partial dried meats with slurry of defatted groundnut paste and spices before a second period of sun drying and partial roasting. It is prepared in both dry and rainy seasons (Igene, *et al.*, 1990; Musonge and Njolai, 1994).

2.3.1 Effects of packaging materials and storage on *Kilishi* and meat products

The type of packaging material is a key factor in ensuring the meat product protection. App.ropriate packagings of meat product eliminate air and subsequently lengthen its shelf life. Ogunsola and Omojola (2008) in their study evaluated the effect of polyvinyl chloride, aluminium foil and plastic container packaging materials on microbial isolates in *Kilishi* beef after storage at room temperatures. The Polyvinyl chloride packed *Kilishi* was reported to be contaminated with *Bacillus* and *Staphylococcus* spp,

aluminium foil packed *Kilishi* encouraged the growth of *Bacillus*, *Streptococcus aureus* and *Streptococcus* spp, while only *Yeast* was isolated from the plastic container package. Mgbemere *et al.* (2011) reported that contamination of stored meat products occurred from the air, environment and other medium during its packaging.

Idowu *et al.* (2010) reported decreased in fungal count from 8 x 10^2 cfu/g in produced *Kilishi* to 4 x 10^2 and 3 x 10^2 cfu/g after storage of the *Kilishi* at 27 and 50 °C respectively. These authors reported that the reduction in microbial population during storage was attributed to the lower moisture content during storage at elevated temperature condition and the spices used in the production of the samples. Oladejo and Adebayo-Tayo (2011) reported increased in ash content in preserved dried beef known as "Banda" after storage, while Faleye and Fagbohun (2012) reported increased in crude protein content in preserved dried beef known as "Tinco" after storage. In these "Tinco" meats, it was reported decreased in moisture, fat and fibre contents after storage. After storage, these authors also reported increased in Na, K and Mg contents and decreased in Ca, Zn and Fe contents.

2.3.2 Effects of ingredients on *Kilishi* and meat products

Studies have shown that the quality of *Kilishi* produced by the traditional processors varies from one producer to the other and from one batch to another from the same producer due to lack of standardized ingredient mix and unit operation that would ensure consistent product quality (Igene, 1988; Olusola, 2006).

Badau *et al.* (1997) reported that the major part of protein in *Kilishi* comes from the groundnut cake, which has 55.85%. However, Olusola *et al.* (2012) reported that the groundnut paste used for the production of the *Kilishi* did not significantly affect the product quality. Mgbemere *et al.* (2011) reported that the conventional defatted groundnut flour *Kilishi* contained high values of moisture, protein and fat than the a locally defatted groundnut cake paste known as "Tunkusa" *Kilishi* (12.1 and 11.6% moisture, 51.8 and 49.8% protein, 13.4 and 11.4% fat respectively). In their findings, it was reported that the conventional defatted groundnut flour *Kilishi* had lower values of ash, crude fibre and carbohydrate contents than "Tunkusa" *Kilishi* (5.1 and 5.2% ash, 2.8 and 3.1% crude fibre and 14.8 and 18.9% carbohydrate respectively).

Though all ingredients contribute their quota of protein in *Kilishi* product, Otunola *et al.* (2010) reported the presence of mineral elements such as Ca, K, P, Na, Mg, Cu and Zn analyzed in garlic, ginger and pepper. In their study, they concluded that these spies used in *Kilishi* production contributed significantly to its quality. Badau *et al.* (2013) reported that groundnut flour, onion and sweet pepper also contained Ca, Fe, Mn, Mg, P, K, Zn and Cu in different proportions.

In their study, Olusola *et al.* (2012) reported that ginger, African-nutmeg and garlic had effect on fat content by lowering the fat considerably and the spices contributed to the ash content of the finished products. De-wit *et al.* (1978) reported that fresh garlic extract in meat product inhibits the growth of *Escherichia coli*, *Shigella dysenteriae*, *Salmonella* Typhi and *Staphylococcus aureus*, while Saleem and Al-Delaimy (1982) reported the inhibition of *Bacillus cereus* by garlic extracts.

2.4. Proximate Composition of Meat Products

The proximate composition generally means the percent composition of five basic constituents such as water, protein, lipid, ash and carbohydrate in food substances (Pushparajan *et al.*, 2012). Protein, lipid and carbohydrate contribute to the total energy content; water and ash contribute to the mass while the total energy content of a specimen can be extrapolated from its proximate composition (Nielsen, 1998).

Moisture content is one of the most commonly measured properties of food material. It gives an idea about the microbial stability, food quality and food-processing operations like mixing, drying, flow through pipe system or packaging (Nielsen, 1998). Isa and Okubanjo (2012) reported that the presence of the various infusion ingredients had no effect on the moisture retention of the meat. The importance of lipid content determination in food include to ascertain its economic value and to confirm if the nutritional labelling conforms to the standard identity, as well as health implications and ability to be processed, as processing depends on total lipid content (Proctor and Meullenet, 1998). The composition of fat in *Kilishi* significantly determines it tenderness and roughness as well as be responsible for its juiciness and sweet aroma upon cooking as reported by Adeniyi *et al.* (2011). Meat usually contains high levels of complete protein, which are normally easy for most people to digest. From nutritional point of view, meats importance is derive from its high quality protein, containing all essential amino acids and its highly bio available minerals and vitamins (Pearson, 1981).

Ash is the inorganic residues remaining after the water and organic matter have been remove by heating in the presence of oxidising agents, which provides a measure of the total amount of minerals within a food. Ash content is a measure of the amount of

mineral present within a given sample of food (Nielsen, 1998). According to Ogunsola and Omojola (2008), the increase in ash content in meat product could be due to adhering dirt on the roasting medium and an indication of the individual mineral levels of the spices respectively. Dietary fibre content is generally define as the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine (Brummer and Cui, 2005). Carbohydrates are either simple or complex, and are major sources of energy in all human diets. Carbohydrate analysis was devised to separate carbohydrates into two broad classifications: crude fibre and nitrogen free extract (Lloyd *et al.*, 1978). Olapade *et al.*, (2004) stated that crude fibre content as well as carbohydrate in meat products contributed by flour, spices and condiment used in their production.

Igene *et al.* (1993) reported that *Kilishi* contained 7.5% moisture, 18% fat, 9.8% ash, 50% protein and 9.8% fibre. Idowu *et al.* (2010) reported that the *Kilishi* analysed contained 9.75% moisture, 18.10% fat, 7.10% ash, 57.20% protein and 8.05% carbohydrate in *Kilishi* meat. Faleye and Fagbohun (2012) reported from *Tinco* dried beef products 17.50% moisture, 17.15% fat, 2.05% ash, 69.98% protein and 4.08% carbohydrate.

2.5 Mineral Composition (Macro Elements) of Meat Products

Minerals are the building blocks of our bodies that are required for body structure, fluid balance, protein structures, hormones producers and act as co-factors, catalysts or inhibitors of all enzymes in the body (Wilson, 2014). Various factors, such as the concentration of minerals in the diet of an animal, hormones, age, gender and region, may cause variations in the mineral composition of its meat (Doyle, 2007).

2.5.1 Sodium (Na)

Table salt (sodium chloride) is the main dietary source of sodium. Other sources include meat, processed foods, fish, etc. Sodium aids in muscles contraction and nerve transmission. According to Griffith (1988), salt acts as a preservative through growth inhibition and destruction of microorganisms by dehydrating meat tissue thereby lowering the water activity. Sodium (Na) is a systemic electrolyte that is crucial in maintaining blood pressure and is essential in co-regulating ATP with potassium. Excess Na causes electrolyte imbalance, which is an indication of high level of sodium known as hypernatremia while insufficient Na causes hyponatremia (Balch *et al.*, 1990). Shereen (2015) reported that excess Na in the body could lead to vomiting and hypertension. According to Institute of Medicine (2003), dietary Na intake is 2,300 mg/day. The Committee on Dietary Allowance Food and Nutrition Board (1989) recommended 1500 and 2,200 mg/day dietary consumption of Na for adolescence and adult respectively. Williams (2007) recorded in red meats from Australia Na contents that ranged from 51.00 to 71.00 $mg100g^{-1}$, while Francis (2007) recorded Na content ranged between 490.00 and 714.00 mg100g-1 in bush meat products. Faleye and Fagbohun (2012) reported Na contents ranged from 29.00 to 33.50 $mg100g^{-1}$ in *Tinco* dried beef products.

2.5.2 Calcium (Ca)

Calcium is found in meat, dried peas and beans, milk, etc. Ca is necessary for the building of strong bones and teeth, blood clotting and vital for nerve and muscles functions (Balch *et al.*, 1990). Saeed (1998) reported that Ca is responsible for strong bones and teeth but its high level in human is responsible for thirst, increased volume of

urine, muscle fatigue, poor mental concentration and formation of kidney stones. According to the Institute of Medicine (2003), Ca intakes for males (1year and older) ranges from 870 to 1,300 mg/day and for females is 750 to 970 mg/day. The Committee on Dietary Allowance Food and Nutrition Board (1989) recommended Ca intake 800 to 1200 mg/day. Jones *et al.* (2001) reported Ca contents (55.69 $mg100g^{-1}$) while Ogbonnaya and Linus (2009) recorded 54.69 $mg100g^{-1}$ Ca in *Kilishi* samples respectively. Faleye and Fagbohun (2012) found that Ca concentration in *Tinco* dried beef beef from Lagos, Nigeria ranged between 20.66 and 24.10 $mg100g^{-1}$.

2.5.3 Magnesium (Mg)

Meat is a good source of Mg and other sources include raw leafy green vegetables, nuts, soya beans and grains (Mayer and Jeanne, 1990). Magnesium is an essential element needed for healthy bones and blood vessels, and aids in muscle function and nerve transmission (Balch *et al.*, 1990). High or low level of Mg causes serious problems including kidney failure and heart problems (Saeed, 1998). While Mg is essential mineral to human, excess of it causes hypermagnesemia. Hypermagnesemia is rare from sources of food because the kidney can work to rid the body of it in the urine (Mayer *et al.*, 1990). The Recommended Dietary Allowances (RDA) of Mg is 300 and 400 mg/day (Institute of Medicine, 2003). Ogbonnaya and Linus (2009) recorded Mg content of 114.49 $mg100g^{-1}$while Jones *et al.* (2001) recorded Mg content of 123.98 $mg100g^{-1}$ in *Kilishi* respectively. Faleye and Fagbohun (2012) reported Mg contents of ranged 28.00 to 30.42 $mg100g^{-1}$ in *Tinco* dried beef products.

2.5.4 Phosphorus (P)

Phosphorus (P) is an important element for many essential processes in the body. In combination with calcium, P is necessary for the formation of bones and teeth, and is involved in the metabolism of fat, carbohydrate and protein, and in the effective utilization of many of the B-group vitamins and in energy metabolism (Göncü *et al.*, 2008). Foods that are rich in protein are excellent sources of phosphorus. These include meat, poultry and fish as well as milk and other dairy products.

The amount of phosphorus needed in a diet depends on the age of the consumer. The Committee on Dietary Allowance Food and Nutrition Board (1989) recommended 700 and 1500 mg of P dietary consumption for adult (19 years and older) and children (9 to 18 years) respectively. The RDA of phosphorus proposed by Jean and Goldberg (1990) is 800 - 1500 mg/day. Ferreira *et al.* (2000) reported that phosphorus contents in meat products from Brazil ranged between 123.56 and 396.96 $mg100g^{-1}$. Jones *et al.* (2001) reported 392.42 $mg100g^{-1}$ phosphorus and Ogbonnaya and Linus (2009) reported phosphorus content of 392.42 $mg100g^{-1}$ *Kilishi* respectively.

2.5.5 Potassium (K)

Potassium is involved in nerve function, muscle control and blood pressure. Göncü *et al.* (2008) reported that a diet low in potassium and high in sodium might be a factor in high blood pressure. Although K is involved in nerve function, muscle control and control of blood pressure but when accumulated due to kidney failure to rid excess K in the body (hyperkalemia), it may lead to irregular heartbeat or heart failure (Mayer *et al.*, 1990; Institute of Medicine, 2003). The RDA of potassium proposed by Mayer *et al.*

(1990) is 1875 - 5625 mg/day. However, there are no any reports of K toxicity from food (Shereen, 2015). Osborne *et al*. (1996) and Shills (1992) also reported that diet that contained high value of K than Na, can provide protection against hypertension in people who are sensitive to high levels of sodium. Francis (2007) in his studies of bush meats reported K contents range between 38.00 and 730.00 $mg100g^{-1}$ while Williams (2007) reported potassium contents range between 344.00 and 365.00 $mg100g^{-1}$ in red meat products. Faleye and Fagbohun (2012) reported that *Tinco* dried beef contained K levels between 33.57 and 47.37 $mg100g^{-1}$.

2.6 Heavy Metals and their Toxicity in Meat and Meat Products

The term "heavy metal" was in use as far back as 1817 when Gmelin divided the elements into non-metals, light metals and heavy metals (Nielen and Marvin, 2008). Heavy metals are those metals whose densities fall within 5.308 - 22.000 g/cm^3 (Hawkes, 1997). Its common sources are from mining and industrial wastes; vehicle emissions; lead-acid batteries; fertilizers; paints and treated woods (Nielsen, 1982). It was reported by Shi *et al.* (2008) that Pb, Zn and Cu mainly originated from traffic contaminants; soil Ni was associated with natural concentration; Cd largely came from point-sourced industrial pollution; and Cr, Ni in dust were mainly related to atmospheric deposition.

Heavy metals in general have been classified as toxic (lead, cadmium, mercury, arsenic) and essential (cobalt, copper, zinc, iron) (Mukesh *et al.*, 2008). A toxic metal is defined as that metal, which is neither essential nor has beneficial effect and on the contrary, it displays severe toxicological symptoms even at low levels (Akan *et al.*, 2010). Generally, long-term exposure to heavy metals can lead to several diseases like

cardiovascular, tubular dysfunction in kidneys and nervous disorders due to metal toxicity (Friberg *et al.*, 1986 and WHO, 1996).

2.6.1 Iron (Fe)

Iron is the next most abundant metal in the globe after aluminium and the important iron ores are magnetite (Fe_3O_4), hematite (Fe_2O_3) and iron pyrites (FeS_2) (Narayanan, 2007). Iron rusts in humid air and corrodes intensively in water that makes it an undesirable component of drinking water (Harvey and Champe, 1994). Major sources of iron pollution are mining, acid mine drainage, corroded metal, domestic and industrial waste (Narayanan, 2007). It has been reported that Fe found in meats is attached to hemoglobin, and is among the most easily absorbed and used Fe of any dietary sources (Saeed, 1998). Iron (Fe) is necessary for helping oxygen molecules bind to red blood cells, and meat is an excellent source of this mineral (Mayer *et al.*, 1990). Iron deficiency in humans shows in the premenopausal woman (Syed *et al.*, 2003). Low iron gives symptoms of fatigue and low energy while excessive iron can cause damage to organs such as the liver, heart and pancreas.

The recommended dietary permissible limit of Fe is 80 mg/day (FAO/WHO, 1997). The upper tolerable intake level of Fe in children and adult is 40 and 45 mg/day respectively recommended by Institute of Medicine (2002). Amani and Lamia (2012) in Saudi Arabia recorded the levels of Fe contents ranged between 68.7 and 290.0 μgg^{-1} and between 175.69 and 242.44 μgg^{-1} in meat and meat products respectively. Dalia and Bassma (2015) in Egypt recorded Fe concentrations in beef meat and meat products of ranging from 82.95 to 352.90 ppm and from 82.90 to 270.00 ppm respectively.

2.6.2 Copper (Cu)

The major portion of copper produced in the world is use by the electrical industries and most of the remainder is combined with other metals to form alloys (Prusty, 1983). Important series of alloys in which copper is the chief constituent are brasses (copper and zinc) and bronzes (copper and tin) (Morse, 1994). The corrosion of copper containing alloys in pipefittings may add measurable amount of copper into the water (Syed *et al.*, 2003). Sources of Cu contamination in foods include copper water pipes, meats using copper sulfate as a growth enhancer, alcoholic beverages from copper brewery equipment, pesticides, insecticides, fungicides, copper cooking pots (Victor *et al.*, 2013). In addition, high concentration of copper oxide may result from welding operation.

Copper and zinc are the cofactors to many enzymes and a key role of Cu in human health is due to its involvement in absorption, utilization and transport of Fe in the body, and so, prevents against the Fe deficiency (Jarzynska and Falandysz, 2011). In the nervous system alone, copper toxicity is associated with migraine headaches, premenstrual syndrome, depression, anxiety, panic attacks, some schizophrenia, seizures, and more (Wilson, 2014). Excess accumulation of copper in the liver might result in hepatitis or cirrhosis (Johnson, 1993). Copper deficiency results in anemia. The congenital inability to excrete copper results in accumulation and 'wilsons', S disease (Greenwood and Earnshaw, 1986). According to WHO/FAO (1980), the maximum Cu concentration for meat and meat products consumption is 8.0 $mgkg^{-1}$. Zahurul *et al.*, 2011) reported Cu levels in meat products ranging between 0.75 and 165.02 $mgkg^{-1}$ while

Amani and Lamia (2012) reported Cu levels range between 11.11 and 18.51 μgg^{-1} in meat products.

2.6.3 Zinc (Zn)

Zinc metal is use in galvanizing iron and steel against corrosion and in making brasses and alloys for die-casting (Narayanan, 2007). The main sources of Zn pollution in the environment are industrial waste, metal plating and plumbing (Narayanan, 2007).

The toxicity of Zn is because of its excessive absorption that suppresses Cu and Fe absorption (FAO/WHO, 2011). Zinc salts are intestinal irritants and can cause nausea, and abdominal pain (ATSDR, 2002). Prolonged exposure to high intakes of Zn results in copper deficiency and subsequent anaemia (Reilly, 2002). It has been reported that Zn is able to damage nerve receptors in the nose, which can cause anosmia and recommended that consumers should stop using zinc-based intranasal cold products and ordered their removal from store shelves (Johnson *et al*., 2007; Safty *et al*., 2008). According to Kline *et al.* (2010) and ATSDR (2004), too much of Zn is harmful to human health, while too little of it can result in poorly developed sex organs and retarded growth in young men and babies (if pregnant women do not have enough zinc). However, a higher amount of Zn is required to overcome the toxic effects of Cadmium, because Zn replaces Cd in many enzymes (Khan *et al.*, 1990).

The permissible limit of Zn in food according to Australia-New Zealand Food Authority (ANZFA, 2001) is 150 mg/L. The Central Agency for Standardization and Quality Control of Iraq (1988) recommended that Zn should not exceed 50 $mgkg^{-1}$ in luncheon meat. Akan *et al.* (2010) discovered in beef samples the levels of Zn that ranged

from 3.87 to 4.24 $\mu g g^{-1}$. Zahurul *et al.* (2011) found in meat products levels of Zn between 17.20 and 284.65 $mg kg^{-1}$ while Faleye and Fagbohun (2012) reported Zn content range between 0.71 and 3.01 $mg 100g^{-1}$ in *Tinco* dried beef products.

2.6.4 Cobalt (Co)

Natural sources of cobalt to the environment include volcanic eruptions, seawater spray and forest fires (Narayanan, 2007). Anthropogenic sources of cobalt to the atmosphere include coal-fired power plants and incinerators, and exhaust from vehicles (Morse, 1994).

Cobalt is a trace element essential in nutrition and an integral component of vitamin B_{12} found mostly in meat and dairy products (Harvey and Champe, 1994). Cobalt can accumulate to toxic levels in the liver, kidney, pancreas and heart as well as the skeleton and skeletal muscles, and it has been found that Co produces tumors in animals and is likely a human carcinogen as well (Narayanan, 2007). The recommended dietary intake of vitamin B_{12} (Co) is 6.00 µg (0.006 $mg kg^{-1}$) (FAO/WHO, 1997). Akan *et al.* (2010) reported a mean value of 0.23 $\mu g g^{-1}$ of cobalt in beef samples while Zahurul *et al.* (2011) recorded Co concentrations in meat products ranging between 0.01 and 48.30 $mg kg^{-1}$.

2.6.5 Manganese (Mn)

Most of the manganese (Mn) in the air is due to the burning of fossil fuels (Syed *et al.*, 2003). Depending on their oxidation state, Mn ions have various colours and therefore are use industrially as pigments, while Mn is use as the cathode material in standard and alkaline disposable dry cells and batteries (Zhang and Cheng, 2007).

Manganese pollution (Mn-oxides) is mainly due to metallurgy, ferrous metal industry and industrial wastes (Narayanan, 2007).

Mn, which in animal cells is associated as an enzymatic cofactor in mitochondria, takes a role in the regulation of cell metabolism, receptor binding and signal transduction pathways (Santamaria and Sulsky, 2010). Upper tolerable intake of Mn for people is 2 - 11 mg/day (Anonymous, 2002). Although it is a required trace mineral for all known living organisms, Mn can cause poisoning syndrome in mammals, with neurological damage, which is sometimes irreversible (ATSDR, 2002). People exposed to high levels of environmental pollution by Mn suffer from cerebella dysfunctions, neurological damage (Reilly, 2002). Akan *et al.* (2010) reported that meat of beef samples from Borno, Nigeria contained Mn of range between 1.04 and 1.34 $\mu g g^{-1}$. Amani and Lamia (2012) reported the concentration of Mn in meat and meat products from Saudi Arabia to range from 11.79 to 32.67 $\mu g g^{-1}$.

2.6.6 Lead (Pb)

Lead is present in several minerals but all are of minor significance except the sulphide, PbS (galena), which is the major source of lead production throughout the world (Taylor, 1995). Lead is obtained from its sulphide mineral as in anglesite ($PbSO_4$) and cerussite ($PbCO_3$) (Alain 1967). Pb, in meat is a toxic metal of primary concern, present at low concentrations in most foods. Contaminations of food during processing or food production in contaminated areas are the main reasons for enhanced Pb intake via foodstuffs. Sources of Pb contamination of food include air, water, food ingredients and

spices, cooking utensils and food packaging. Results of the spices used to produce *Kilishi* meats showed that spices might be source of Pb contamination (Gupta *et al.*, 2003).

Lead would become toxic, if consumed in the amount that exceeds the threshold and can cause acute and chronic poisoning symptoms (Jarup, 2003). The Commission of the European Communities (2002) reported that Pb reduced cognitive development and intellectual performance in children and increased blood pressure and cardiovascular disease in adults. US Environment Protection Agency reported that Pb might be implicated in causing leukemia (Anonymous, 2002). FAO/WHO (2001) and EC, Commission Regulation (2001) established limits for Pb content of 0.3 mg/kg (fresh weight basis), i.e., 1.44 mg/kg dry weight. According to FAO/WHO (2000) the recommendation consumer dietary intake of Pb is 0.5 mg kg^{-1}.Demirezen and Uruc (2006) recorded Pb values between 11.5 and 13.5 $\mu g100g^{-1}$ in meat and meat products from Turkey. Akan *et al.* (2010) found Pb concentration ranging between 0.15 and 0.25 μgg^{-1} while Bendeddouche *et al.* (2014) reported Pb ranging between 7.76 and 5.85 μgg^{-1} in beef samples respectively.

2.6.7 Cadmium (Cd)

Cadmium is a non-essential toxic metal that has no biological role, found as an environmental contaminant, both through natural occurrence and from industrial and agricultural sources (Andree *et al.*, 2010). Its human toxicity occurs through food chain magnification and foodstuffs are the main source of Cd exposure for the non-smoking general population (Andree *et al.*, 2010). Cadmium occurs in a few minerals and in small quantities in other ores, especially zinc-ores from which it is produce as a by-product

(Wedelpohl, 1998). Wilson (2014) reported that cigarette and marijuana smoke increase cadmium toxicity a lot. It is widespread in the air, as it is use in brake linings of cars and in metal plating as it is a very hard substance. According to Waegeneers *et al.* (2009), Cd concentrations in meat samples have an increasing trend with age, there is a significant positive linear relation between animal age, and renal or hepatic Cd levels.

It has been reported that Cd absorption after dietary exposure in humans is relatively low, but it is efficiently retained in the kidney and liver in human body, with a very long biological half-life ranging from 10 to 30 years (Andree *et al.*, 2010; Waegeneers *et al.*, 2009). Cd can also cause bone demineralization, either through direct bone damage or indirectly because of renal dysfunction. It has been classified as a human carcinogen and has been listed as endocrine disturbing substance that may lead to the development of prostate and breast cancer (Burger and Gochfeld, 2000; EFSA, 2009; Pan *et al.*, 2010). Toxic effects of cadmium are kidney dysfunction, hypertension, hepatic injury and lung damage and can be fatal in cases of acute poisoning (John and Jeanne, 1994; Burger and Gochfeld, 2000).

According to EC (2001), the permissible limit for Cd consumption is 1.0 $mgkg^{-1}$. FAO/WHO (2000) recommends Cadmium toxicification under the maximum permissible concentration of 0.5 $mgkg^{-1}$). Akan *et al.* (2010) reported Cd concentration in beef of ranged 0.17 to 0.22 μgg^{-1}. Amani and Lamia (2012) recorded in meat and meat products Cd levels ranging from 0.83 to 2.02 and from 3.06 to 4.08 μgg^{-1} respectively.

2.6.8 Chromium (Cr)

Chromium has density of 7.2 gcm^{-3} and is the 21st most abundant element in Earth's crust with an average concentration of 100 mg/L (Emsley, 2001). It is used in metal alloys such as stainless steel, protective coatings of metal (electroplating), magnetic tapes, and pigments for paints, cement, paper, rubber and its soluble form is used in wood preservatives as well as additive in water to prevent corrosion in industrial and other cooling system (Hingston, 2001; WHO, 2003). Food is the main source of Cr intake by man. Chromium is fairly distributed throughout the various food groups.

As reviewed by some authors (Wardlaw, 1999; Bae *et al.*, 2000; Institute of Medicine, 2002), Cr has been reported as an essential element for human. It is associated with biologically active Cr (naturally occurring trivalent Cr (Cr III), while hexavalent Cr (Cr VI) does not occur naturally) as a cofactor of insulin which is involved in glucose metabolism as well as in lipid and protein metabolism. Too little chromium in the diet may lead to insulin resistance (Institute of Medicine, 2002). An adequate Cr intake is 25 µg per day for adults and between 0.1 and 1.0 µg for children and adolescents as quoted by EFSA (2009). According to FAO/WHO (1984), the maximum permissible daily intake of Cr is 0.10 mg. Demirezen and Uruc (2006), reported Cr concentration ranged between 8.44 and 9.51 $\mu g100g^{-1}$ in meat and meat products. Akan *et al.* (2010) recorded Cr mean levels in beef samples of range between 0.32 and 0.43 μgg^{-1}.

2.6.9 Nickel (Ni)

Nickel with density of 8.9 g/cm^3 readily forms alloys with iron, aluminium, zinc, molybdenum, and copper and can be dissolve in dilute acids (Reilly, 2002). About 60%

of world production of Ni is use in nickel-steels, particularly stainless steel (Obasohan, 2008). Syed *et al.* (2003) reported that vehicles exhaust a large quantity of Ni from the petroleum used. In their report, it was said that cigarette smoking increases the inhaled Ni to as much as 4 µg per pack of cigarette.

According to ATSDR (2004), Ni can contribute to cancer and other horrible problems such as respiratory problem. Among the known health-related effects of Ni are skin allergies, lung fibrosis, variable degrees of kidney and cardiovascular system poisoning and stimulation of neoplastic transformation (Kasprzak *et al.*, 2003; Nussey *et al.*, 2000). The upper tolerable intake level of nickel for children (1 - 3 years old) and adults (19 - 70 years old) is 7 and 40 mg/day respectively (Institute of Medicine (2002). The permissible limit of Ni consumption in food is 0.5 mg kg^{-1} (FAO, 1995). Demirezen and Uruc, 2006) reported that the concentration of Ni in meat and meat products from Turkey ranged from 8.2 to 24.0 µg$100g^{-1}$. Akan *et al.* (2010) reported Ni levels of ranged 0.16 to 0.29 µgg^{-1} in beef samples from Nigeria.

2.7 Microbiological Effect of Meat and Meat Products

Meat, which is a rich source of the protein and fat, low in carbohydrate content and with sufficient water activity, supp.orts the growth of both spoilage and pathogenic bacteria, thus classified by Food Codex in high-risk food (Komba *et al.*, 2012). According to Food Safety and Inspection Services, USDA (2001), beef is at risk of three organisms' contamination: *E. coli*, *Salmonella*, and *Listeria monocytogenes*. In the United States, the food vehicle for 41% food borne outbreaks found was from ground beef (Schroeder *et al.*, 2005). Sources of meat contamination as reported by Galland

(1997) depend on the respective animal feeds such as polluted water and crops grown on irrigated sewerage water and industrial effluents. It also depends on the deposition of contaminants from dust, vehicle emission and dirty slaughter places. In addition, 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 (Dorsa *et al.*, 1995). It is cause by the practically unavoidable infection and subsequent decomposition of meat by bacteria and fungi, which are borne by the animal itself, the people handling the meat or the implements used during processing (Lawrie and Ledward, 2006). According to FAO/WHO (2000), microbial contamination of meat and meat products when exceeded its permissible level could adversely affect the shelf life of the product. It renders the meat useless, unwholesome and hence not fit for human consumption.

2.7.1 Mesophillic aerobic count

Aerobic plate count is a measure of microbial quality of the meat and meat products and must not exceed 10^6 cfu/g or cm^2 (Pearson, 1986). Reports showed that pathogenic bacteria such as *Salmonella* spp., *Bacillus cereus* could contaminate food including its products, and the contamination levels are influenced by climatic conditions (Blackburn, 2006; Richer *et al.*, 1993). Adetunde *et al.* (2011) in Ghana reported total viable bacterial counts that ranged between 2.12×10^7 and 3.26×10^7, and between 4.16

$\times 10^7$ and 9.97×10^7 cfu/g in beef from slaughter yard and open market respectively. In their study, it was concluded that the variations of microbial count could be due to the condition in which animals are being kept and improper handling of the animal.

2.7.2 *Staphylococcus*

Microbiologically, Staphylococci are characterized as gram-positive, non spore-forming, non-motile and facultative anaerobes, which are universally present in great numbers (Norrung *et al.*, 2009). *S. aureus* is a common species of *Staphylococcus* strains, which are major agents of wound and human skin infections and are one of the most common causes of food poisoning (FAO/WHO, 2001). It was also been reported that *S. aureus*, one of the family of *Staphylococcaceae* cause meningitis, pneumonia, urinary tract infections and mastitis. The contamination standard of *S. aureus* set by FAO/WHO (2001) is 10^2 cfu/g. According to Benneth (1981), curing salt (sodium chloride or sodium nitrate or sodium nitrite) and subsequent proper handling method might favours the growth of gram-positive bacteria, primarily *Staphylococcus aureus*, while inhibiting the proliferation of gram-negative bacteria. Laciakova *et al.* (2004), studied in Slovak Republic reported 2.2×10^3 to 2.8×10^4 cfu/g of *S. aureus* in meat products. Odey *et al.* (2013) in Calabar, Nigeria reported *S. aureus* counts of 21.43% in *Kilishi* and concluded that the presence of *Staphylococcus* spp affirmed the cross contamination through processing, since it is a normal flora of the skin.

2.7.3 Coliform

Coliform bacteria are characterized as rod-shaped gram-negative non-spore forming and motile or non-motile bacteria, which can ferment lactose with the production

of acid and gas (Frazier and Westhoff, 1978). The typical genera of Coliform bacteria include *Citrobacter*, *Enterobacter*, *Klebsiella*, *E. coli*, among others. *E. coli* is a bacterium that is always present in the intestines of humans and animals and in food, which indicates faecal contamination or hygiene quality of the food (Frazier and Westhoff, 1978). Similarly, Frazier and Westhoff (1978) reported that the presence of fecal Coliform of 30% in a sample indicates a high risk that other pathogenic organisms have also contaminated the sample. A standard of 10^2 cfu/g coliform was stated by FAO/WHO (2001). Laciakova *et al.* (2004) in Slovak Republic recorded Coliform count in *Kilishi* meats (3.2×10^3 to 1.8×10^4 and 3.2×10^2 to 8.2×10^6 cfu/g) prepared in winter and summer months respectively. Odey *et al.* (2013) in Calabar, Nigeria reported Coliform counts ranged from 1.0×10^2 to 3.5×10^2 cfu/g in meat and meat products.

2.7.4 Fungi

Meat and meat products having intermediary moisture content, has been reported as a potential substrate for fungal development that is caused by contamination that can occur after processing, during packaging and inapp.ropriate storage (Blackburn, 2006). Lawrie and Ledward (2006) reported that spoilage of meat by *Moulds* in the presence of oxygen produced sticky and "whiskery" surface, discoloration, change in odour and fat decomposition symptoms. As reported by many authors (Wu *et al*, 1989; Zaky *et al.*, 1995; Berwal, 1991), numerous strains of microscopic filamentous fungi, isolated from the surface of various meat products, show in vitro the ability to produce toxic substances. The presence of *Moulds* in the meat products usually causes a metabolic interaction with various bacterial pathogens and therefore, can participate in an outbreak of food-borne illness (Norrung *et al.*, 2009). Mycotoxin producing fungi such as

Aspergillus spp., *Penicillium* spp. *and Fusarium* spp. can easily contaminate food and their contamination levels are influenced by climatic conditions (Blackburn, 2006; Richer *et al.*, 1993). Fonkem *et al.* (2010) in Cameroon reported fungi counts in *Kilishi* meats of 0.15 and 1.21 cfu/g in dry and rainy seasons respectively. The contamination standard of fungi is 10^3 cfu/g (FAO/WHO, 2001).

CHAPTER THREE

3.0 METHODOLOGY

3.1 Study Area

The study areas are Zaria and Kaduna towns in Kaduna State of Nigeria. Zaria town town covers an area of approximately 563 km^2 and is bounded by latitude 11^o 04' 00" N to 11^o 07' 00" N and longitude 7^o 42' 00" E to 7^o 70' 00" E of Northern Kaduna State (Figure 3.1). The indigeneous Hausa predominantly occupy Zaria town neighbourhood. Kaduna town covers approximately 131 km^2 and the area is bounded by latitude 10^o 31' 23" N to 10^o 52' 30" N and longitude 70^o 26' 25" E to 70^o 44' 28" E (Figure 3.2). It is the state capital of Kaduna State in northwestern Nigeria. The town is an industrial centre of Northern Nigeria manufacturing products like textiles, petroleum products, etc. The study areas has tropical wet and dry (savanna) climate with a pronounced dry season that lasts from November to mid-April and a rainy season that lasts from mid-April to November. The State extends from the tropical grassland known as the Guinea Savannah to Sudan Savannah. These two climatic conditions greatly influenced activities of the predominantly occupied people, who are primarily based on agriculture. It also favours animal keeping. In addition, the population of Nigerians recorded in Kaduna and Zaria towns of 760,084 and 695,089 respectively (2006 census) influenced business activities. Thus, *Kilishi* business is prominent in Kaduna and Zaria towns of Kaduna State.

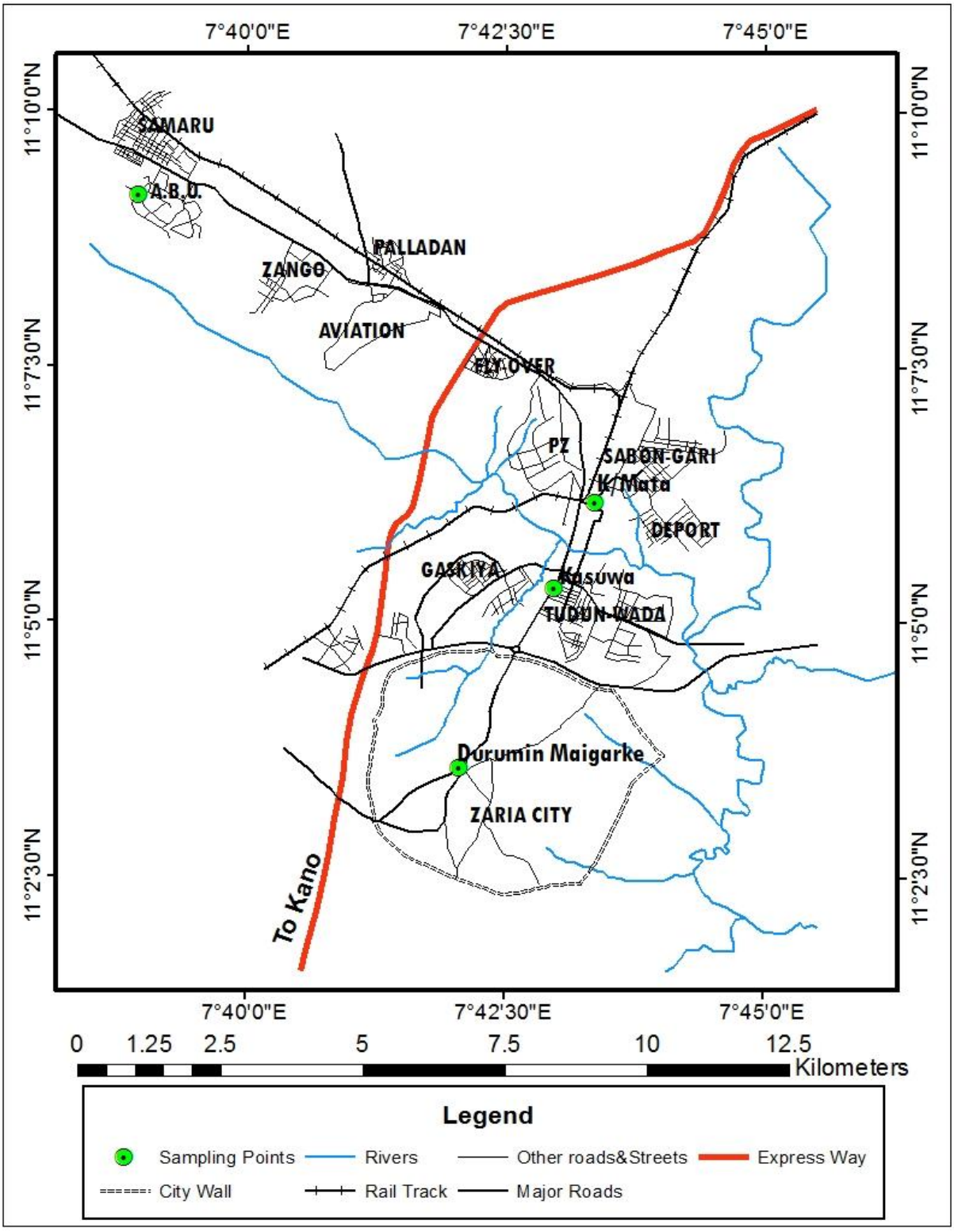


Figure 3.1: Map of Zaria Metropolis (Town) Showing Sampling Points

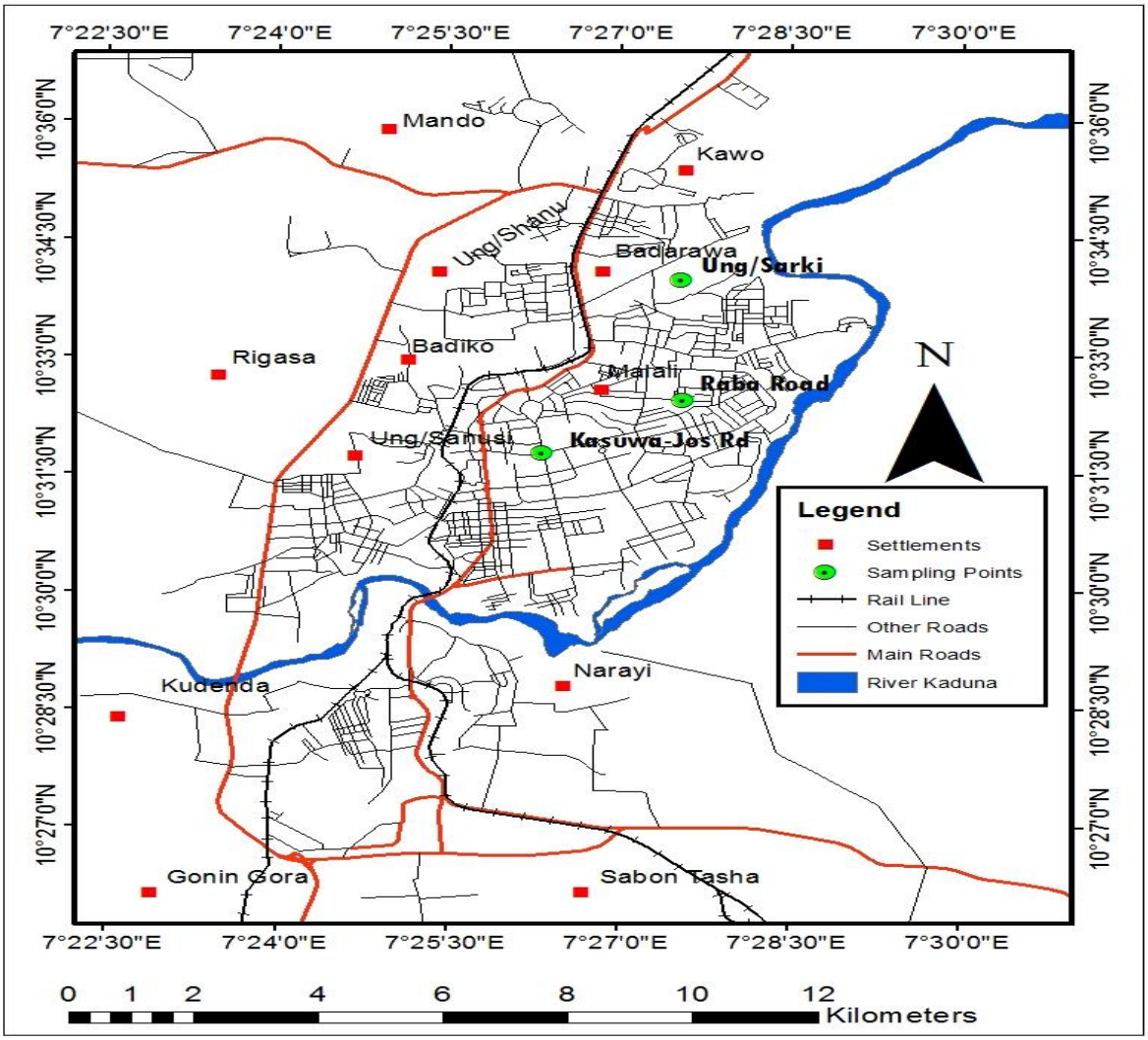


Figure 3.2: Map of Kaduna Metropolis (Town) Showing Sampling Points

3.2 Location of the Experiment

The experiment was carried out at the Multi-User Science Research Laboratory, Departments of Food Science, Soil Science and Microbiological Science of Ahmadu Bello University, Zaria; located within the Northern Guinea Savannah Zone, latitude 11^0 $90^{'}$ $45^{''}$ N and Longitude 7^o 38' 80'' E as shown in figure 3.1 above.

3.3 Materials Procurements and *Kilishi* Preparations

Raw beef was purchased from Zangon Shanu abattoir in Zaria, Kaduna State, Nigeria. Exactly 2 kg of fresh beef each were distributed to six commercial *Kilishi* producers, at their respective *Kilishi* production spots in Zaria axis and Kaduna town and were used to prepare *Kilishi* meat. The Zaria axis study sites were Sabon Gari-Kasuwan Mata, Tudun Wada-Kasuwa and Zaria city-Durumin Maigarke (Figure 3.2), while Kaduna town study sites were Malali-Rabah Road, Anguwan Sarki-Sultan Bello Road and Kasuwa- Jos Road (Figure 3.3). Similarly, 2 kg of fresh beef was prepared as Laboratory *Kilishi*. The spice ingredients used for the Laboratory *Kilishi* were purchased individually from specialized ingredients shops in Samaru and Sabon Gari markets, Zaria. All reagents used in this research work were of high analytical grades.

3.3.1 Preparation of *Kilishi* by commercial producers

The ingredients formulations, infusion time, duration of the solar drying stages due to environmental conditions and final roasting stage time differed amongst the commercial producers. *Kilishi* preparation by the commercial producers involved:

i. The fat was trimmed and removed from the fresh beef.

ii. The trimmed meat was cut into flat thin sliced sheets using a sharp knife.

iii. The thin sheets were spread out on raffia mats and sundried (first stage drying), to reduce the moisture content and condition the meat for ingredient infusion.

iv. The pre-dried meat was infused with the ingredient mixed powder slurry comprises *Tunkusa*, spices and condiments ground into powder form.

v. The infused ingredients mixed slurry sheets were again spread out on raffia mats and allowed to dry (second stage drying)

vi. After the second stage drying, the products were finally roasted in a smokeless glowing fire.

vii. The resultant *Kilishi* product was then wrapp.ed with Newspaper for the analysis.

3.3.2 Beef preparation

Exactly 2 kg of fresh semitendonosus muscle from hot boned beef was trimmed of fat and excess connective tissues. The resultant lean beef, which weighed 1.8 kg was then cut into smaller portions of 200 g and sliced into thin sheets of 2 mm thick. These pieces of the sliced meat were thinly spread out on a cleaned stainless steel wire mesh and oven dried at a temperature of 50 ^{o}C for 4 hours. During the drying process, the meat slices were turned-over after every hour to allow for an even drying, which prevented the sliced meats from been stocked to the drying surface. The weight of each sliced meat was recorded before and after this first stage drying, while the pre-dried meats were packaged in aluminum foil and kept in an airtight container for the next step in processing.

3.3.3 Groundnut paste preparation

Exactly 5 kg of groundnut seeds were mildly roasted in an air oven at 100 ^{o}C for 5 minutes. The roasted seeds were dehulled by hand abrasion, winnowed and the hulls

removed. The kernels obtained were oven dried at 55 ^{o}C for 2 hours, milled into flour using kitchen blender, sieved, spread out on a stainless tray and roasted in the oven at 100 ^{o}C for 10 minutes. The resultant flour was defatted by placing the flour in a cleaned plastic bowl and kneading the paste to a maximum pressure to extract the oil (Ogunsola and Omojola, 2008). The defatted flour paste obtained was used for the groundnut paste processing. The defatted groundnut flour paste of 2.8 kg was placed in a cleaned stainless bowl followed by addition of 1.2 kg of the prepared ingredient powder and then 600 cm^3 swan-water. The contents of the bowl were then mixed thoroughly to give a thick-slurry.

3.3.4 Ingredients preparation

All the spices used were mixed together in a specific proportion as shown in App.endix I. The dried ingredients used for the spices powder preparation of 100 g were dried in an air oven at 40 ^{o}C for 4 hours followed by blending with a kitchen blender and then kept for the next step in processing.

3.3.5 Kilishi production, packaging and storage

The pre-dried sliced sheets of the meats were deeply immersed into the prepared groundnut slurry for 30 minutes. Thereafter, the soaked meats were removed and spread out on a cleaned stainless wire mesh and then oven dried at 50 ^{o}C for another 4 hours, with constant turning at every thirty minutes. The dried-infused meats were mildly roasted at 60 ^{o}C for 10 minutes in an oven and then allowed to cool at room temperature. The products obtained were packaged in aluminum foil and stored in desiccators for three days before analysis.

3.4 Sample Labeling

Each sample of the oven-dried raw beef, oven-dried *Kilishi* prepared in the Laboratory of Department of Chemistry, Ahmadu Bello University, Zaria and six samples of sun-dried *Kilishi* prepared from selected *Kilishi* production spots in Kaduna State were labeled as follows:

i. RB: Oven-dried raw beef prepared in the Laboratory.
ii. CL: Oven-dried *Kilishi* prepared in the Laboratory.
iii. SG: Sun-dried *Kilishi* from Kasuwan Mata, Sabon Gari, Zaria
iv. TW: Sun-dried *Kilishi* from Tudun Wada, Zaria
v. ZC: Sun-dried *Kilishi* from Durumi Maigarke, Zaria City, Zaria
vi. MK: Sun-dried *Kilishi* from Raba Road, Malali, Kaduna
vii. UK: Sun-dried *Kilishi* from Anguwan Sarki, Kaduna
viii. KK: Sun-dried *Kilishi* from Jos Road, Kasuwa, Kaduna.

The samples collected were dried in an oven at 60 ^{o}C for 4 hours in the Laboratory of Department of Chemistry, Ahmadu Bello University, Zaria. A portion of each dried labeled sample was finely ground into powder using ceramic pestle and mortar, packaged and stored in polyethylene bags for further analysis.

3.5 Determination of Proximate Parameters

3.5.1 Moisture content (AOAC, 1980)

A cleaned crucible was oven dried at 100 ^{o}C and allowed to cool to a constant weight (W_1) in a desiccator. Two grams of the sample was placed into the crucible and

the weight (W_2) noted. The crucible and its content (W_2) were oven dried at 100 ^{o}C for eight hours and allowed to cool in a desiccator. The drying procedure was repeated to a constant weight (W_3) and the percentage moisture content was calculated using equation 3.1 below.

$$\% \text{ Moisture} = \frac{W_2 - W_3}{W_2 - W_1} \times 100 \qquad 3.1$$

3.5.2 Crude fat (AOAC, 1980)

A finely ground sample of 5.0 g was accurately weighed and semi-dried to a constant weight before being subjected to ether-extraction. The dried sample was placed in an extraction thimble of weight W_1, and its total weight, W_2 was noted. The thimble and the content was placed into a cleaned and dried 250 cm^3 capacity round bottomed flask fitted into a soxhlet extraction unit and 100 cm^3 of petroleum ether (40 – 60^{o}) was then poured into the extraction unit containing the sample and some anti-bumping granules were added. The extraction was carried out at 40 ^{o}C for 8 hours with a drip rate of 3 drops per second after which the heating mantle was switched off.

After the extraction, the extract was transferred into a clean glass dish with thorough ether washings after which the ether was evaporated on a water bath. The extract was then placed in an oven set at 70 ^{o}C for 2 hours after which was allowed to cool in a desiccator and its weight W_3 was recorded. The percent crude fat extract was then calculated using equation 3.2 below.

$$\% \text{ Crude fat} = \frac{W_2 - W_3}{W_2 - W_1} \times 100 \qquad 3.2$$

3.5.3 Crude protein (Kjeldahl method: AOAC, 1990)

Principle: Determination of protein in the samples is by Kjeldahl method. The samples are been digested by heating with concentrated sulphuric acid (H_2SO_4) in the presence of digestion mixture [Potassium sulphate (K_2SO_4) and copper sulphate ($CuSO_4$)]. The digestion converts any nitrogen in the food into ammonia, and other organic matter to CO_2 and H_2O. The ammonia that will be release, which is in the form of ammonium ion (NH_4^+), will bind to the sulfate ions (SO_4^{2-}) and thus remains in solution:

$$\text{N (food)} \longrightarrow (NH_4)_2SO_4 \qquad 3.3$$

The mixture was made alkaline by addition of sodium hydroxide, which converted the ammonium sulfate into ammonia gas:

$$(NH_4)_2SO_4 + 2NaOH \longrightarrow 2NH_3 + 2H_2O + Na_2SO_4 \qquad 3.4$$

Ammonium sulphate thus formed, released ammonia, which was collected in 2% boric acid solution and titrated against standard HCl solution.

$$NH_3 + H_3BO_3 \text{ (boric acid)} \longrightarrow NH_4^+ + H_2BO_3^- \text{ (borate ion)} \qquad 3.5$$

$$H_2BO_3^- + H^+ \longrightarrow H_3BO_3 \qquad 3.6$$

The nitrogen concentration of the sample was determined and the amount of protein was calculated by multiplying the amount of nitrogen with an app.ropriate factor of 6.25.

Preparation of reagents:

Sodium sulphate and anhydrous copper sulphate catalyst (1:10): This was prepared by mixing accurately 10.0 g of sodium sulphate (Na_2SO_4) with 100.0 g of anhydrous copper (II) sulphate ($CuSO_4$), which formed a mixed catalyst.

Sodium hydroxide solution (40%): This solution was prepared by dissolving 40.0 g of sodium hydroxide, NaOH (F.W 40) in distilled water, after which the solution was carefully transferred into a 100 cm^3 volumetric flask and then made up to the mark.

Boric acid solution (40%): This was prepared by dissolving 4.0 g of boric acid (H_3BO_3) in a 100 cm^3 volumetric flask containing distilled water and then made up to the mark.

Hydrochloric acid solution (0.1M): This was prepared by transferring 7.58 cm^3 concentrated hydrochloric acid, HCl (Sp. gr. 1.19, 37.2%) using a micropipette into a 100 cm^3 volumetric flask. The solution was then made up to the mark with distilled water.

Methyl red indicator (0.1 wt. of solution in water): This was prepared by dissolving approximately 0.4 g of methyl red as sodium salt in 40.00 cm^3 ethanol (64%) and then bringing the solution to 100 cm^3 with distilled water.

Procedure:

Digestion: The digestion of the sample was carried out by placing 2.0 g of finely ground meat sample into a dried Kjeldahl flask after which 1.0 g of the prepared mixed catalyst was added, followed by 6 cm^3 of concentrated H_2SO_4 and some anti-bump granules. The mixture was heated for 4 hours, until a greenish solution that turned to colourless was observed. The colourless solution after cooling was made up to 100 cm^3 in a volumetric flask with distilled water.

Distillation: The distillation of the prepared digested solution was carried out by placing 10 cm^3 of it into a Kjeldahl distillation app.aratus. Then, 10 cm^3 of 40% sodium hydroxide solution was added, which liberated ammonia gas. The ammonia gas liberated was then collected in a 100 cm^3 conical flask containing 5 cm^3 of 40% boric acid and 2 drops of methyl red indicator. The collection of the distillate was continued until the pink colour of the indicator turned greenish.

Titration: The content of the conical flask were titrated with 0.1M HCl solution to an end-point which was indicated by a change from green to pink colour and the titre volume noted. The procedure was repeated three times and the average titre value was calculated. The titre value for the blank solution was also noted. The percent nitrogen, N and crude protein were then calculated, using equations 3.7 and 3.8 below.

$$\% N = \frac{(VA - VB) \times M \times 1.40}{W} \qquad 3.7$$

$$\% \text{ Crude protein} = \% N \times 6.25 \qquad 3.8$$

Where VA = titrant for standard acid (cm^3)

VB = titrant for blank (cm^3)

M = molarity of acid

W = weight of sample in grams

1.40 = molecular weight of nitrogen in kilogram per liter

6.25 = conversion factor for protein (equivalent to 0.16 g N/g of protein)

3.5.4. Ash content (AOAC, 1980)

A cleaned empty crucible was placed in a muffle furnace at 200 ^oC for an hour. It was cooled in desiccator and its weight W_1 was recorded. A defatted sample of 2.0 g was placed in the weighed crucible and the weight W_2 was recorded. The sample in the crucible was ignited over a burner with the help of blowpipe, until it was charred. The crucible containing the charred content was then placed in a muffle furnace set at 500 ^oC and allowed to ash for 4 hours. The app.earance of grey white ash indicated complete oxidation of all organic matter in the sample. The crucible was then cooled and weighed (W_3) and the percent ash was calculated, using equation 3.9 below.

$$\% \text{ Ash} = \frac{W_2 - W_3}{W_2 - W_1} \times 100 \qquad 3.9$$

3.5.5 Crude fibre (AOAC, 1980).

Principle: Determination of fibre is by enzymic-gravimetric method. It involves enzymic treatments for starch and protein removal. After then, precipitation of the soluble fibre components by aqueous ethanol, carry out isolation and weighing of the dietary fibre

residue. From the final weight of the dietary fibre residue, there would be correction for protein and ash values.

Preparation of Reagents:

Sulphuric acid solution (0.023M): An aliquot volume of concentrated sulphuric acid (Sp.gr. 1.84; 96.0%) of 1.28 cm^3 was accurately transferred into a 1000 cm^3 volumetric flask containing some amount of distilled water and the solution was then made up to mark.

Sodium hydroxide solution (0.312M): Exactly 12.50 g of pellets, NaOH (Sp.gr. 1.01, F.W. 40.0) was accurately weighed and dissolved in about 250 cm^3 distilled water. The solution was accurately transferred into a 1000 cm^3 volumetric flask and made up to the mark with distilled water.

Procedure: This experiment was carried out by placing 2.0 g of a moisture-free and deffated sample (W) into a round bottomed flask where 100 cm^3 of 0.023M H_2SO_4 solution was added, and the mixture was boiled under reflux for 30 minutes. The hot solution was quickly filtered under suction while the insoluble materials were washed several times with hot water until it was acid free. The washed residue was placed into a flask and another portion of 100 cm^3 of hot 0.312M NaOH solution was added. The contents were boiled for another 30 minutes, quickly filtered under suction and the insoluble residues washed with boiled water until it was base free.

The weight obtained of the base free-residue (W_1) was recorded. The residue was dried in an oven set at 100 ^oC, with constant cooling in a desiccator to a constant weight (W_2). This residue was then incinerated in a muffle furnace at 550 ^oC for 2 hours and the

weight of the ash (W_3) was recorded after cooling in a desiccator. The percent crude fibre was then calculated, using equation 3.10 below.

$$\% \text{ Crude fibre} = \frac{W_2 - W_3}{W} \times 100 \qquad 3.10$$

3.5.6 Carbohydrate content (AOAC, 1980; Pearson, 1981)

The carbohydrate content (CHO) was determined by difference, using equation 3.11 below.

% CHO = 100 – (% moisture + % ash + % crude protein + % crude lipid) 3.11

3.6 Determination of Mineral Contents and Heavy Metals (AOAC, 2000)

3.6.1 Preparation of reagents and aqueous stock solutions

Nitric acid and hydrochloric acid solution (1:3 v/v): This was prepared by accurately transferring 100 cm^3 of concentrated nitric acid, HNO_3 (Sp.gr: 1.42; 70.0%) into a 300 cm^3 of hydrochloric acid (Sp.gr. 1.19, 37.2%) in a beaker.

Perchloric acid and nitric acid solution (1:4 v/v): This solution was prepared by accurately transferring 400 cm^3 of concentrated perchloric acid, $HClO_4$ (Sp.gr. 1.06, 69.0%) into a volumetric flask, after which 100 cm^3 of concentrated nitric acid (70.0%) was carefully added.

Nitric acid (3%): A given volume (251.7 cm^3) of concentrated nitric acid (Sp.gr. 1.42, 70.0%) was accurately weighed and then transferred to about 800 cm^3 distilled water in a 1000 cm^3 volumetric flask. The solution was then made up to mark after cooling.

Ammonium metavanadate solution (2.5 g/L): Exactly 2.5 g of dried ammonium metavanadate, NH_4VO_3 was dissolved in 500 cm^3 warm water and allowed to cool. The solution was transferred into a 1000 cm^3 volumetric flask, 20 cm^3 of concentrated HNO_3 (Sp.gr. 1.42, 70.0%) was added to it and was diluted to the mark.

Molybdate solution, $(NH_4)_2MoO_4$ (0.5M): Exactly 72.0 g of anhydride molybdenum trioxide (MoO_3) was dissolved in a mixture of 60 cm^3 of concentrated ammonium hydroxide, NH_4OH (Sp.gr: 0.90; 56.6%) and 200 cm^3 deionized water. The solution was filtered and the filtrate was poured slowly, with constant stirring into an already prepared mixture of 270 cm^3 concentrated HNO_3 acid (70.0%) and 400 cm^3 deionized water. The solution was then diluted to 1000 cm^3 and allowed to stand for 8 hours at 60 °C, after which a yellow-colour precipitate of ammonium phosphomolybdate complex was formed. The solution was decanted from the sediment into glass-stopp.er containers.

Colour reagent (developer for phosphate determination): One volume each of concentrated nitric acid (Sp.gr: 1.42; 70.0%) and the prepared ammonium monovanadate solutions were mixed together in a beaker respectively. After then, one volume of the prepared 0.5M ammonium molybdate solution was added and a clear reagent was obtained.

Phosphorus solution (1000 mg/L): This solution was prepared by dissolving 4.39 g of anhydrous potassium dihydrogen phosphate (KH_2PO_4) in distilled water and diluting to 1000 cm^3.

Sodium chloride solution (1000 mg/L): This was prepared by dissolving 0.51 g of sodium chloride (NaCl) in 200 cm^3 deionized water.

Calcium solution (1000 mg/L): This solution was prepared by dissolving 0.25 g of dried calcium carbonate ($CaCO_3$) in 20 cm^3 deionized water followed by addition of 10 cm^3 of 6M HCI solution. The solution was then diluted to 1000 cm^3 with deionized water.

Magnesium solution (1000 mg/L): Exactly 1.10 g of dried magnesium oxide (MgO) was dissolved in deionized water followed by addition of 10 cm^3 of 6M HCI solution. The solution was then diluted to 1000 cm^3 with deionized water.

Potassium solution (1000 mg/L): This solution was prepared by dissolving 1.91 g of dried potassium chloride (KCl) in 250 cm^3 of deionised water and then making the solution to mark in a 1000 cm^3 volumetric flask.

Iron solution (1000 mg/L): Exactly 4.84 g of dried dehydrated iron (11) chloride tetrahydrate ($FeCl_2.4H_2O$) was dissolved in 200 cm^3 deionised water and the solution was then made to mark in a 1000 cm^3 volumetric flask.

Copper solution (1000 mg/L): This was prepared by dissolving 3.80 g of dehydrated copper nitrate hemipentahydrate ($Cu(NO_3)_2.2.5H_2O$) in 5 cm^3 concentrated HNO_3 solution (70.0%) and was made up mark with deionized water.

Zinc solution (1000 mg/L): This solution was prepared by dissolving 1.25 g of zinc oxide (ZnO) in 5 cm^3 deionized water, after which 25 cm^3 of 5M HCl was added and the solution was diluted to 1000 cm^3 with deionized water.

Cobalt solution (1000 mg/L): Exactly 4.04 g of dried cobalt (11) chloride hexahydrate ($CoCl_2.6H_2O$) was dissolved in 200 cm^3 deionized water and the solution was then made to mark in a 1000-cm^3 volumetric flask.

Manganese solution (1000 mg/L): This solution was prepared by dissolving 3.61 g of dried manganese (II) chloride tetrahydrate ($MnCl_2.4H_2O$) in 50 cm^3 concentrated HCl (37.2%) and then diluting the contents to 1000 cm^3 with deionized water.

Lead solution (1000 mg/L): Exactly 1.60 g of lead (II) nitrate ($Pb(NO_3)_2$ was dissolved in 100 cm^3 deionized water and the solution was then diluted to 1000 cm^3.

Cadmium solution (1000 mg/L): This solution was prepared by dissolving 1.63 g of cadmium chloride ($CdCl_2$) in 250 cm^3 deionized water and then diluting the solution to 1000 cm^3.

Chromium solution (1000 mg/L): Exactly 2.83 g of dried anhydrous potassium dichromate ($K_2Cr_2O_7$) was dissolved in 250 cm^3 deionized water and then made up to 1000 cm^3 mark.

Nickel solution (1000 mg/L): Exactly 4.95 g of dried nickel (II) nitrate hexahydrate ($Ni(NO_3)_2.6H_2O$) was dissolved in 250 cm^3 deionized water after which the solution was diluted to 1000 cm^3.

3.6.2 Digestion of samples

3.6.2.1 Dry digestion of samples (AOAC, 1980)

Two (2) g of ground sample was placed each in a crucible dish. The crucible dish containing the sample was then placed in a temperature controlled Muffle furnace and the furnace was heated at 300 ^oC for 2 hours, until the sample materials were dried and charred. After charring, the temperature of the furnace was increased and held at 500 ^oC for 4 hours where the charred sample turned to white ash. The ash sample was removed

and cooled in a desiccator. The ash obtained was quantitatively transferred to a 100-cm^3 beaker and was dissolved in 10 cm^3 of concentrated mixed acid (HCl : HNO_3 1 : 3 v/v) for digestion. The digested solution was then evaporated off at 150 °C on an electric hot plate to near dryness and the residue obtained was solubilized with 20 cm^3 of 3% HNO_3, which was heated for 5 minutes. The contents were filtered through Whatman No. 44 filter paper into a 100 cm^3 volumetric flask. After allowing the filtrate to cool its volume was made to mark with deionized water, labeled accurately and then stored for mineral determination. The digestion of each sample was carried out alongside with the blank.

3.6.2.2 Wet digestion of samples (AOAC, 1980)

This was carried out by placing 2.0 g of each ground sample in a 100 cm^3 digestion glass tube followed by the addition of 10 cm^3 of a mixture of 70% high purity HNO_3 and 65% $HClO_4$, 4:1 v/v. The mixture was was then heated to boiling gently at 80 °C until white fumes were evolved and allowed to cool. After cooling, 2 cm^3 of 30 % hydrogen peroxide was added to the contents, which was reheated until a transparent solution was obtained. The heating was increased and the content, which evaporated to about 1 cm^3 was allowed to cool. To the cooled content, 25 cm^3 of 3% HNO_3 solution was added and the mixture was reheated for 1 hour and allowed to cooled. The heating was repeated after addition of another portion of 25 cm^3 of 3% HNO_3. The solution obtained was filtered hot through Whatman filter paper into a 100 cm^3 volumetric flask, allowed to cool and the filtrate made to mark with deionized water. The wet digested solution was accurately labeled and stored for mineral determination. The digestion of each sample was carried out alongside with the blank.

3.6.3 Preparation of calibration curves

Six working standard solutions were prepared for each metal by serial dilution of stock solution. These standards and the blank solution were aspirated in the atomic absorption spectroscopic instrument and their absorbance measured. Then the calibration curves of absorbance versus concentrations of each element were plotted.

3.6.4 Instrumental parameters for atomic absorption spectroscopy (AAS)

The wavelength, current, flow rate, slit width and burner height for the atomic absorption spectroscopy (AAS) used for the elemental analysis of this study are presented in Appendix II.

3.6.5 Determination of sodium (Na) and potassium (K) (AOAC, 1984)

Principle: Sodium and potassium metals were determined by flame photometry. The flame photometer measures the emission of radiant energy when atoms of an element return to their ground state after their excitation by the high temperature of the flame. The degree of light intensity relates to the concentration of the element in the solution.

Procedure: Na and K analysis of the sample were carried out by the method of flame photometry. Aliquot volumes of each sample from wet digested solutions were introduced into a flame photometer (Jenway model PF9 flame photometer) and the concentrations of Na and K metals were analyzed respectively. This was carried out at Department of Soil Science, Ahmadu Bello University, Zaria.

3.6.6 Determination of phosphorus (P) (AOAC, 1984).

Principle: Phosphorus was determined by colorimetric method. Colorimetric determination is based upon the principle that certain elements or compounds on reaction with suitable reagent developed colour. The intensity of the color is measure with colorimeter or spectrophotometer. The inorganic phosphorus reacts with ammonium molybdate and ammonium phosphomolybdate formed, which on reaction with the light intensity produces molybdenum blue. The blue color of the solution is now measured and the amount of the phosphorus is determined.

Preparation of test sample: A mixture of an aliquot amount of the prepared sample solution (taken to contain 300 µg cm^{-3} Phosphorus and 70 cm^3 of molybdate solution) was digested at 65 °C for 1 hour to form precipitate. The precipitate formed, was dissolved in 25 cm^3 of 0.1M NH_4OH solution and then diluted to 100 cm^3 volume with deionized water.

Procedure: From the prepared test sample, 1.0 cm^3 of it was placed into a plastic bottle and 4.0 cm^3 distilled water was added. Afterthen, 5 cm^3 of color reagent was added to the diluted solution in the plastic bottle and the mixture (final solution) was made up to 25.0 cm^3. After some time, the color of this final solution turned blue. An aliquot volume of the final blue solution in a cuvett that contained the sample was placed in a Model 253 Corning Colorimeter. Then the absorbance of the coloured complex was measure against standard phosphate solutions and the readings of the phosphorus were recorded in mg/L. This analysis was carried out at Department of Soil Science, Ahmadu Bello University, Zaria.

3.6.7 Determination of heavy metals (AOAC, 1984)

Principle: The concentrations of Ca, Mg, Fe, Cu, Zn, Co, Mn, Pb, Cd, Cr and Ni were determined by atomic absorption spectrometry. In this technique, atoms of an element are vaporized and atomized in the flame. The atoms then absorb the light at a characteristic wavelength. The source of the light is a hollow cathode lamp, which is made-up of the same element, which has to be determined. The lamp produces radiation of an appropriate wavelength. The free atoms of the sample absorbed the radiation produced by the lamp while it was passing through the flame. The absorbed energy is then measure by a photo-detector read-out system. The amount of energy absorbed is proportional to the concentration of the element in the sample.

Procedure: Samples from dry digested solutions were aspirated and analyzed for heavy metals using Varian AAS 240FS at the Multi-User Research Science Laboratory, Ahmadu Bello University, Zaria.

3.6.8 Calculation of metal concentration

The concentration of each mineral recorded in terms of milligram per litre (mg/L) was then converted to milligram per kilogram (mg/kg) of the mineral, using equations 3.12 and 3.13 below.

$$\text{Mineral (mg)} = \frac{\text{Concentration (mg/L)} \times \text{D}(\text{cm}^3)}{(1000\ cm^3)} \qquad 3.12$$

$$\text{Mineral concentration (mg per kg)} = \frac{\text{Mineral (mg)} \times 1\text{ kg } (1000\text{g})}{\text{M (g)}} \qquad 3.13$$

Where D is volume of solution in cm^3 = 100.00 cm^3

M is the mass of the digested sample in grams = 2.00 g

3.7 Microbiological Procedures (FAO, 1979)

3.7.1 Culture media preparation

The culture media used for the analysis were prepared according to the manufacturers' instructions. The media prepared were Plate Count Agar (PCA) for total Mesophilic aerobic plate count of bacteria, Mannitol Salt Agar (MSA) for Staphylococcus count, Eosine Methylene Blue Agar (EMA) for Coliforms count, Potato Dextrose Agar (PDA) for the enumeration and isolation of fungi (yeasts and moulds) and *Salmonella Shigella* Agar (SSA) for detection of *Salmonella*.

3.7.2 Sample preparation and dilution

Each finely ground sample of 10.0 g was aseptically weighed and homogenized in 90 ml of 0.1% peptone water. A given volume of 1.0 ml of each sample homogenate was diluted to 10 ml with 0.1% peptone water into a first dilution tube (ratio 1:10). From the first dilution solution, 1 ml of the solution was diluted to 10 ml with 0.1% peptone water into the second dilution tube (ratio 1:100). Then, from the second dilution solution, another 1 ml of the solution was further diluted to 10 ml with 0.1% peptone water in the third dilution tube (ratio 1:1000). This serial dilution procedure was carried out at the fouth and fifth dilution tube respectively, which were used for the microbial procedures.

3.7.3 Calculation of microbial count

The microbial count of each sample, in colony forming unit per gram (cfu/g) of each parameter was calculated from the average colony count/plate after incubation as shown in 3.14 below.

$$\text{Microbial count (cfu/g)} = \frac{\text{Total number of colonies counted} \times \text{dilution factor}}{\text{Volume of inocula}} \qquad 3.14$$

3.7.4 Total mesophilic aerobic count

A given volume of 1 ml of each sample homogenate dilutions (10^{-4} and 10^{-5} dilutions) were aseptically transferred into app.ropriately marked sterile duplicate petri dishes, while 15 ml each of previously prepared Plate Count Agar (PCA) was poured into each of the dishes, a process known as pour-plating technique. The sample dilution and agar medium (vortex) on each dish was mixed thoroughly and uniformly and allowed to solidify. These inoculated plates were incubated at 37 ^{o}C for 72 hours. Following incubation, colonies observed on each plate were counted and the formular above (3.14) used to calculate the colony-forming unit per gram (cfu/g).

3.7.5 Enumeration of *Staphylococcus* (S. *aureus*)

A given volume of 0.25 ml of each 10^{-4} and 10^{-5} sample dilutions were aseptically transferred onto appropriately marked surfaces of previously prepared-dried Mannitol Salt Agar (MSA) duplicate plates and then spreaded with a sterile bent glass rod, a process known as spread-plating technique. These inoculated plates were incubated at 37 ^{o}C for 24 hours. After 24 hours, the plates were observed for separate colonies that were black and shiny with white margins, surrounded by cleared zones, presumptive colonies

of *Staphylococcus aureus*. The positions of these colonies were marked and the plates re-incubated for another 24 hours. Colonies that developed during the extended period of incubation were counted and calculated as cfu/g using formular 3.14 above.

3.7.6 Enumeration of coliforms

The procedure for enumeration of coliforms was pour plating (as described under 3.7.4), where 15 ml each of previously prepared Eosine Methylene Blue Agar (EMA) was poured into each of the duplicate dishes containing 1 ml each of the 10^{-4} and 10^{-5} sample dilutions respectively. Each vortex was thoroughly and uniformly mixed and then allowed to solidify. The inoculated plates were incubated at 37 ^{o}C for 72 hours. Following incubation, colonies observed on each plate were counted and then computed as colony forming unit per gram (cfu/g) using the formular above (3.14).

3.7.7 Enumeration of fungi (Yeasts/Moulds)

This was pour-plating procedure as described under 3.7.5, where 15 ml of each prepared Potato Dextrose Agar (PDA) media was poured on each duplicate petri dish containing 1 ml each of the 10^{-4} and 10^{-5} sample dilutions. The inoculated plates were incubated at room temperature for 5 days to observed for fungi colonies that were counted and then calculated as colony forming unit per gram (cfu/g) using the formular above (3.14).

3.7.8 Detection of *Salmonella*

One (1) ml of each prepared homogenate sample dilutions (10^{-4} and 10^{-5} dilutions) was asceptically transferred into appropriately marked sterile duplicate bottles each

containing 9.0 ml of sterile selenite-F broth, an enrichment medium for *Salmonella* species. These enrichment media were then incubated at 37 ^{o}C for 24 hours. After 24 hours, a loopful from each selenite-F broth was streaked unto each previously prepared sterile *Salmonella Shigella* Agar (SSA) duplicate plates. The plates were incubated at 37 ^{o}C for 24 hours and were observed for typical colonies of *Salmonella*.

3.7.9 Identification of bacteria species (Gram stain) (AOAC, 1992)

Principle: Gram staining is normally the first step towards identifying an unknown pathogenic agent. Gram's procedure divides the vast array of bacterial organisms including cocci, Bacilli, and spirilli into two broad groups. Gram staining is a common technique used to differentiate two large groups of bacteria based on their different cell wall constituents. Gram-positive bacteria stain purple or violet, while Gram-negative stain pink or red.

Procedure: A drop of distilled water was placed on each cleaned microscopic slides followed by a colony from each agar plate, which was picked using sterilized wire loop and then emalsified thinly on each slide to prepare a smear. Each smeared slide was air dried and heat-fixed by passing the slide over flame and allowed to cool. It was followed by an addition of Gram's iodine solution, staining with crystal violet, decolorizing with acetone and counterstaining with Safranin. Each Gram stained slide was then examined microscopically under oil immersion (Cheesbrough, 2000).

3.7.10 Biochemical identification of bacterial species (FAO, 1979)

The isolates obtained were subjected to biochemical test for identification using Microgen test kits (Barrow and Feltham, 1993) as detailed below.

Catalase test: This test was used to identify organisms that produce the enzyme, catalase. The enzyme catalase catalyses the breakdown of hydrogen peroxide into water and oxygen gas.

$$2H_2O_2 \longrightarrow 2H_2O + O_2 \qquad 3.15$$

Each drop of an isolate was placed on a cleaned microscopic slide, followed by a drop of 3.5% hydrogen peroxide solution, while the mixture was observed immediately for a bubbling formation from production of oxygen gas that indicated a catalase-positive result of *Staphylococcus aureus*.

Coagulase test: One drop of each prepared test and control latex reagents were placed on appropriately marked surfaces of cleaned reaction cards respectively and each reagent drop was inoculated with 1 ml of isolates. Then, each card was hand rocked for 20 seconds and observed for agglutination under normal lighting conditions that indicated positive identification of *Staphylococcus aureus*.

Indole test: Pure isolates of each bacteria growth were prepared in tryptone broth (indole medium) and incubated at 37 ^{o}C for 48 hours. After incubation, 3 drops of Kovac's indole reagents were added to each indole medium, while the mixture was shaken gently and allowed to stand for 1 minute. The contents was observed for development of a red or pink ring that indicated indole production, a positive identification of *Escherichia coli,* while a negative result that appeared yellow indicated the presence of *Salmonella* spp.

Methyl Red-Vogesproskauer (MR-VP) test: Each pure isolates was inoculated in MR-VP broth media and the media incubated at 37 ^{o}C for 2 days. To 1 ml of each incubated broth in a small serological tube, it was added 3 drops of methyl red reagents and

observed for a red coloration, a positive methyl red results that indicated the presence of *Escherichia coli*. To another 1 ml portion of each broth, it was added a mixture of 0.6 ml alpha-naphthol and 0.2 ml of 40% potassium hydroxide and observed for development of an eosin pink color after 4 hours, a positive result that indicates the absence of *Salmonella* (Harrigan and McCance, 1998).

Citrate Utilization test: A loopful of isolates from each colony was streaked lightly on the surface of each previously incubated prepared Simmons' Citrate Agar Slant (SCAS) using a sterile needle. The Simmons' agar slant contained a pH indicator (Bromothymol blue) and was prepared in a bijou bottle. Each inoculated Simmons' agar slant was incubated at 35 ^{o}C for 24 hours and then observed for pH value, at neutral pH of a green coloration, a negative citrate test result that indicated the presence of *Escherichia coli* or *Salmonella* spp. (Reddy, 2007).

Carbohydrate fermentation test in Triple Sugar Iron Agar (TSI): A loopful of each isolates was asceptically inoculated on each previously incubated prepared TSI slant and butt tubes respectively. Each inoculum was incubated at 37 °C for 8 hours and observed for hydrogen sulfide production and blackening of the medium at the butt tube, and carbohydrate fermentation of a yellow slant of the slant tube (acid and gas production). This positive result indicated the presence of *Salmonella* spp.

Motility test: A loopful of each isolates was asceptically inoculated, by stabbing to a depth of about 5 mm on each prepared motility medium using a sterile inoculating needle. The inoculates were incubated at 35 ^{o}C for 8 hours and were observed for motile organisms that migrated through the medium, which became turbid, indicating the

presence of *Escherichia coli* against *Staphylococcus epidermidis* (without flagella) which growth might be confined to the stab (Health Protection Agency, 2007).

3.7.11 Identification of fungi species (lactophenol cotton blue stain test)

Principle: Lactophenol cotton blue (LCB) staining mounting medium was used for microscopic identification of fungi (moulds and yeasts) species. Its modifications are tested staining mounting medium on parasite positive faecal samples to stain and demonstrate morphology (Cheesbrough, 2000). Organisms suspended in the stain were killed due to the presence of phenol. The high concentration of the phenol deactivated lytic cellular enzymes thus the cells do not lyse. Cotton blue is an acid dye that stains the chitin present in the cell wall of fungi.

Preparation of LCB stain reagent (FAO, 1992): This reagent was prepared over two days: On the first day, 0.05 g cotton blue was dissolved in 20 ml distilled water and left overnight to eliminate any insoluble dye. On the second day, 20.00 g phenol crystals was dissolved in 20 ml lactic acid in a glass beaker using magnetic stirrer after which 40 ml glycerol was added. After then, the two solutions were filtered and mixed into the phenol/glycerol/lactic acid solution. The LCB stain reagent produced was stored at room temperature for the analysis.

Procedure: A loopful of fungi colonies on each incubated Potato Dextrose Agar (PDA) medium were asceptically inoculated into a previously prepared Sabourad Dextrose Agar (SDA) and these enrichment media were incubated at 37 ^{o}C for 3 days. Following incubation, each pure isolates medium was observed for characteristics of moulds and yeast, where large flat colonies with diffused edges and variable colour in the centre

indicated moulds, while small blue-green colonies with defined edges indicated yeast. After then, one drop of LCB stain reagent was placed on a cleaned slide, followed by one drop of each characteristics colony obtained on SDA media, which was spreaded evenly to form the smear. The smeared slide was covered to eliminate any air bubble, after then, it was examined under a light microscope (CETI Belgium, Serial No 060621, ×40 magnifications) for the present of characteristic mycelia and fruiting structures. The black or green colour mycelia (conidophore and conidia) indicated *Aspergillus* spp., the whitish mycelia indicated *Penicillum* spp. while the moist mycelia colony and present of arthroconidia indicated *Geotrichum* spp.

3.8 Environmental Conditions of *Kilishi* Production Spots

The hygienic conditions of the environment at the *Kilishi* production spots were observed. It was observed that the Sabon Gari production spot was located close to welding shops and where chicken are sold and slaughtered. The environment app.eared dirty with stagnant gutters. The meats here were placed on tables, which are not well cleaned after a day's work and in the open with houseflies hovering around. The production spot of Tudun Wada was located close to where goats and rams are sold and slaughtered. Its environment was littered with animals' wastes and bones and was dirty.

It was also observed that the fresh meat were processed on wooden tables that were not neat with houseflies hovering around at all the production spots. The production spots for *Kilishi* from Zaria City, Malali, Anguwan Sarki and Kasuwa were located by the roadsides with vehicles blowing dust on the meat in addition to uncleaned tables used and

houseflies hovering around. In addition, the production spot of Zaria City was located close to a welding shop.

3.9 Statistical Analysis (SAS, 1991)

Results obtained were statistically analyzed using statistical analytical systems (SAS, 1991) package through the following tools:

i. Means of triplicates analysis were computed using ANOVA and standard deviations separated using Duncan Multiple Range Test.

ii. Student t- test was used in ranking means for levels of significant at 95% confident level ($P < 0.05$).

CHAPTER FOUR

4.0 RESULTS

4.1 Proximate Parameters of Dried Beef and its *Kilishi* Samples

The results of the proximate composition of moisture, crude fat, crude protein, ash content, fibre content and carbohydrate content are presented in Table 4.1.

Percentage moisture content was highest in Malali *Kilishi* having 9.23%, which is not significantly different from Zaria City *Kilishi* (8.83%), while it was lowest in dried beef having 6.46% that is not significantly different from *Kilishi* from Kasuwa (7.22%) and Laboratory (6.93%) at $P < 0.05$. The result for crude fat ranged between 17.07 - 22.42%. The highest crude fat value obtained was from Malali *Kilishi* and the lowest value was from the dried beef. For crude protein, the highest value was from dried beef with a value of 67.94% and the lowest value of 52.92% obtained was from Malali *Kilishi*. There is no significant difference of crude protein values observed in *Kilishi* samples from Anguwan Sarki, Laboratory and Sabon Gari ($P < 0.05$). The least percentage of ash obtained was from the dried beef (6.81%) while the highest percent ash was from Anguwan Sarki (9.42%). Tudun Wada *Kilishi* had the highest percent of crude fibre of value 3.42%, while the least value of 0.75% was from the dried beef. The highest percentage of carbohydrate of 8.32% was obtained in *Kilishi* from Zaria City which value has no significant difference from values obtained in Sabon Gari and Malali *Kilishi* ($P < 0.05$), while the dried beef had the lowest carbohydrate value of 1.72%.

Table 4.1: Means and Standard Deviations of the Proximate Composition of Dried Beef and its *Kilishi* Samples

S/C	%Moisture	%Crude fat	%Crude protein	%Ash	% Crude fiber	%Carbohy-Drate
RB	6.46 ± 0.46^{e}	17.07 ± 0.21^{d}	67.94 ± 0.65^{a}	6.81 ± 0.68^{e}	0.75 ± 0.31^{e}	1.72 ± 0.10^{e}
CL	6.93 ± 0.03^{de}	21.37 ± 0.92^{ab}	56.52 ± 0.62^{d}	8.70 ± 0.26^{bc}	1.36 ± 0.02^{cd}	6.48 ± 0.69^{c}
SG	7.88 ± 0.18^{bc}	21.43 ± 0.93^{ab}	55.80 ± 1.38^{d}	6.93 ± 0.05^{e}	1.26 ± 0.07^{d}	7.96 ± 0.58^{a}
TW	7.53 ± 0.63^{cd}	19.27 ± 1.03^{cd}	58.79 ± 0.79^{c}	8.24 ± 0.23^{cd}	3.42 ± 0.04^{a}	6.17 ± 0.62^{c}
ZC	8.83 ± 0.43^{ab}	20.39 ± 1.25^{bc}	53.45 ± 1.42^{e}	9.01 ± 0.08^{ab}	1.29 ± 0.04^{d}	8.32 ± 0.51^{a}
MK	9.23 ± 0.68^{a}	22.42 ± 0.91^{a}	52.92 ± 0.90^{e}	7.79 ± 0.04^{d}	2.24 ± 0.04^{b}	7.64 ± 0.53^{ab}
UK	7.85 ± 0.05^{bc}	17.63 ± 0.99^{d}	57.83 ± 0.14^{cd}	9.42 ± 0.45^{a}	1.34 ± 0.04^{d}	7.27 ± 0.39^{bc}
KK	7.22 ± 0.59^{cd}	17.27 ± 0.35^{d}	64.28 ± 1.73^{b}	6.85 ± 0.16^{e}	1.55 ± 0.04^{c}	4.38 ± 0.57^{d}
M	7.72 ± 0.94	19.61 ± 2.14	58.40 ± 5.04	7.98 ± 1.01	1.28 ± 0.25	6.26 ± 2.17
CV	5.93	7.13	1.95	3.02	1.01	8.16
SE	0.19	0.44	1.03	0.21	0.05	0.44

Key: S/C = sample code, RB = dried raw beef, CL = Lab. *Kilishi*, SG = Sabon Gari *Kilishi*, TW = Tudun Wada *Kilishi*, ZC = Zaria City *Kilishi*, MK = Malali Kaduna *Kilishi*, UK = Anguwan Sarki Kaduna *Kilishi*, KK = Kasuwa Kaduna *Kilishi*, M = total mean, CV = coefficient variance, SE = standard error of means.

a-f: means along the same column with different superscript are significantly different ($P<0.05$)

4.2 Mineral Contents in Dried Beef and its *Kilishi* Samples

The mineral composition (macro elements) in dried beef and its *Kilishi* samples with respect to recommended daily dietary intake are pictorially depict in Figure 4.1, 4.2, 4.3, 4.4 and 4.5 respectively while their Table of results presented in App.endix III. All values of each mineral element in the samples were significantly different from each other at $P < 0.05$ except for phosphorus in Malali and Kasuwa *Kilishi* samples, and potassium from Anguwan Sarki and Laboratory *Kilishi* samples.

Figure 4.1 shows Na contents in *Kilishi* samples ranges from 2,451.00 - 8,281.50 $mgkg^{-1}$ being especially highest in Kasuwa *Kilishi* and lowest in Sabon Gari *Kilishi*. Sodium content in dried beef (785.00 $mgkg^{-1}$) was significantly lower than the values obtained in *Kilishi* samples ($p < 0.05$). Figure 4.2 depicts that the dried beef had the highest Ca content of 564.30 $mgkg^{-1}$, while the lowest Ca value of 337.51 $mgkg^{-1}$ was obtained in *Kilishi* from the Laboratory. Mg content in Fig 4.3 was equally highest in dried beef of value 474.55 $mgkg^{-1}$ and lowest in *Kilishi* from the Laboratory of value 357.51 $mgkg^{-1}$. Phosphorus (P) concentrations of the study samples (Figure 4.4) ranged between 3,015.00 $mgkg^{-1}$ in dried beef and 8,915.00 $mgkg^{-1}$ in Malali *Kilishi*. The Laboratory *Kilishi* had P value of 4,805.00 $mgkg^{-1}$ that was significantly lower than values obtained in the other *Kilishi* samples. Figure 4.5 depicts potassium (K) was highest in Malali *Kilishi* (9,610.00 $mgkg^{-1}$) and lowest in dried beef (5,405.00 $mgkg^{-1}$). *Kilishi* from the Laboratory had K value of 4,005.00 $mgkg^{-1}$ with no significant different to the value obtained from Anguwan Sarki sample ($p < 0.05$).

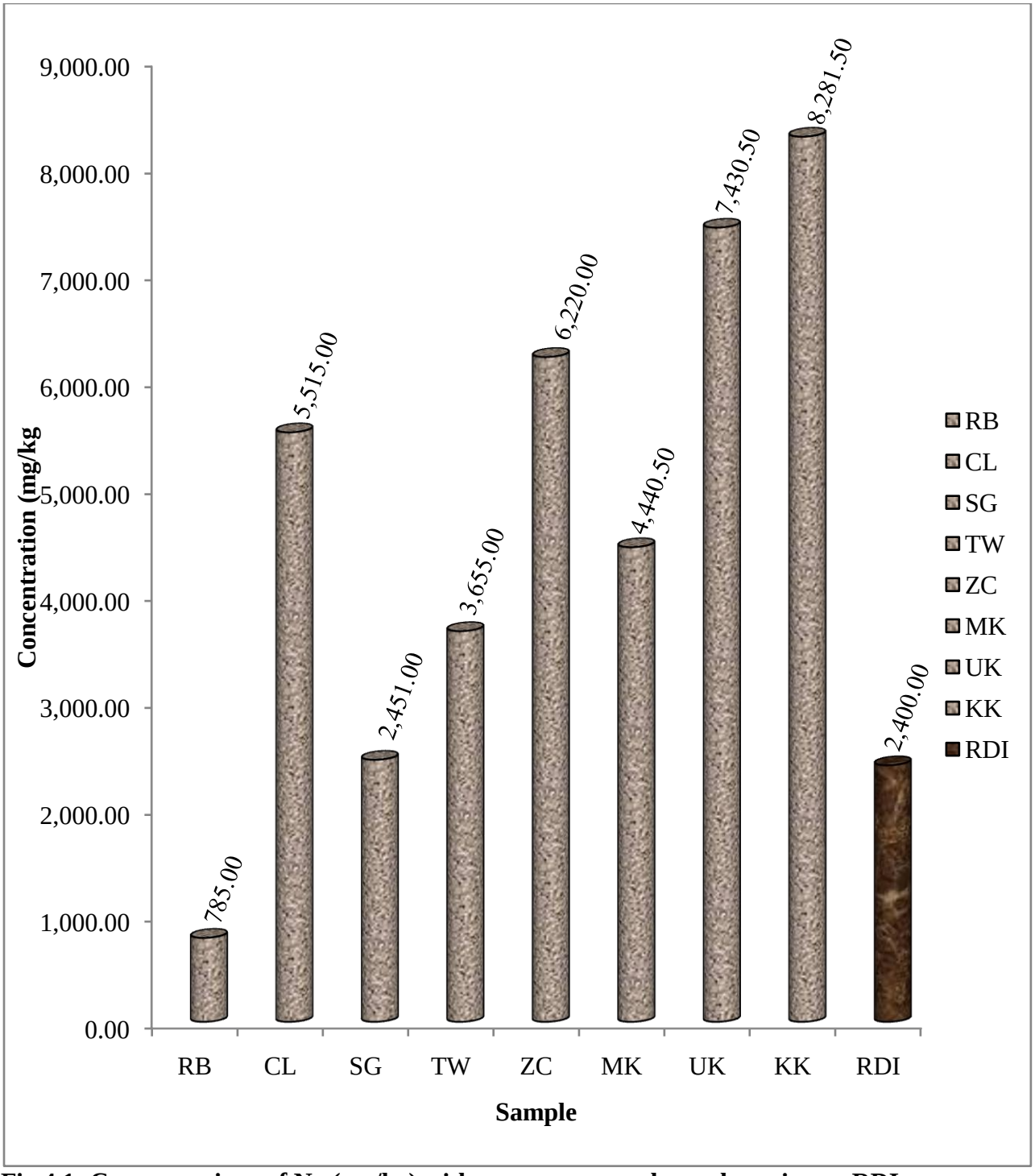


Fig 4.1: Concentrations of Na (mg/kg) with respect to samples and maximum RDI

Key: RB = Dried beef, CL = Laboratory *Kilishi*, SG = Sabon Gari *Kilishi*, TW = Tudun Wada *Kilishi*, ZC = Zaria City *Kilishi*, MK = Malali Kaduna *Kilishi*, UK = Anguwan Sarki Kaduna *Kilishi*, KK = Kasuwa Kaduna *Kilishi* and RDI = Recommended dietary intake.

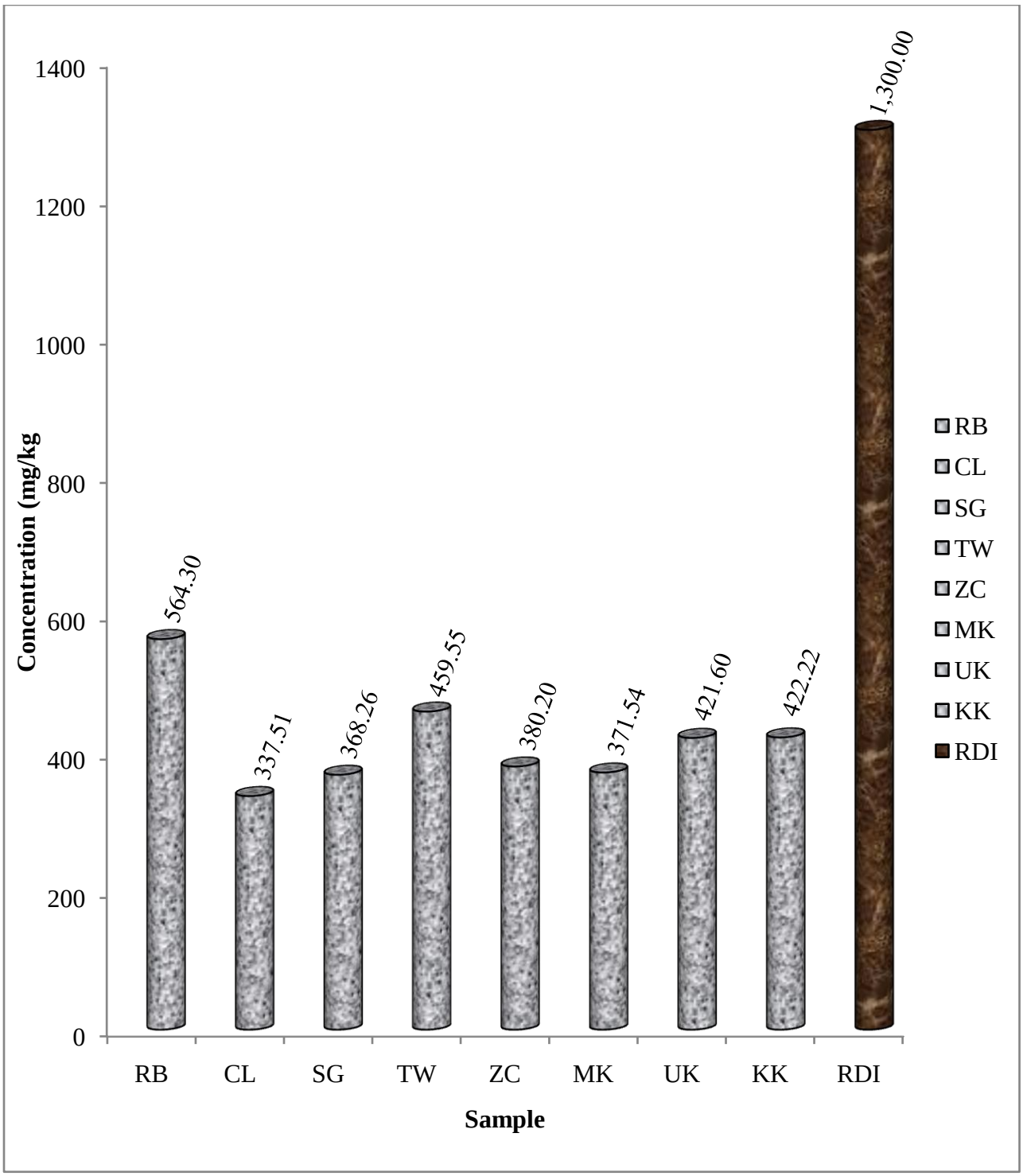


Fig 4.2: Concentrations of Ca (mg/kg) with respect to samples and maximum RDI

Key: RB = Dried beef, CL = Laboratory *Kilishi*, SG = Sabon Gari *Kilishi*, TW = Tudun Wada *Kilishi*, ZC = Zaria City *Kilishi*, MK = Malali Kaduna *Kilishi*, UK = Anguwan Sarki Kaduna *Kilishi*, KK = Kasuwa Kaduna *Kilishi* and RDI =.Recommended dietary intake.

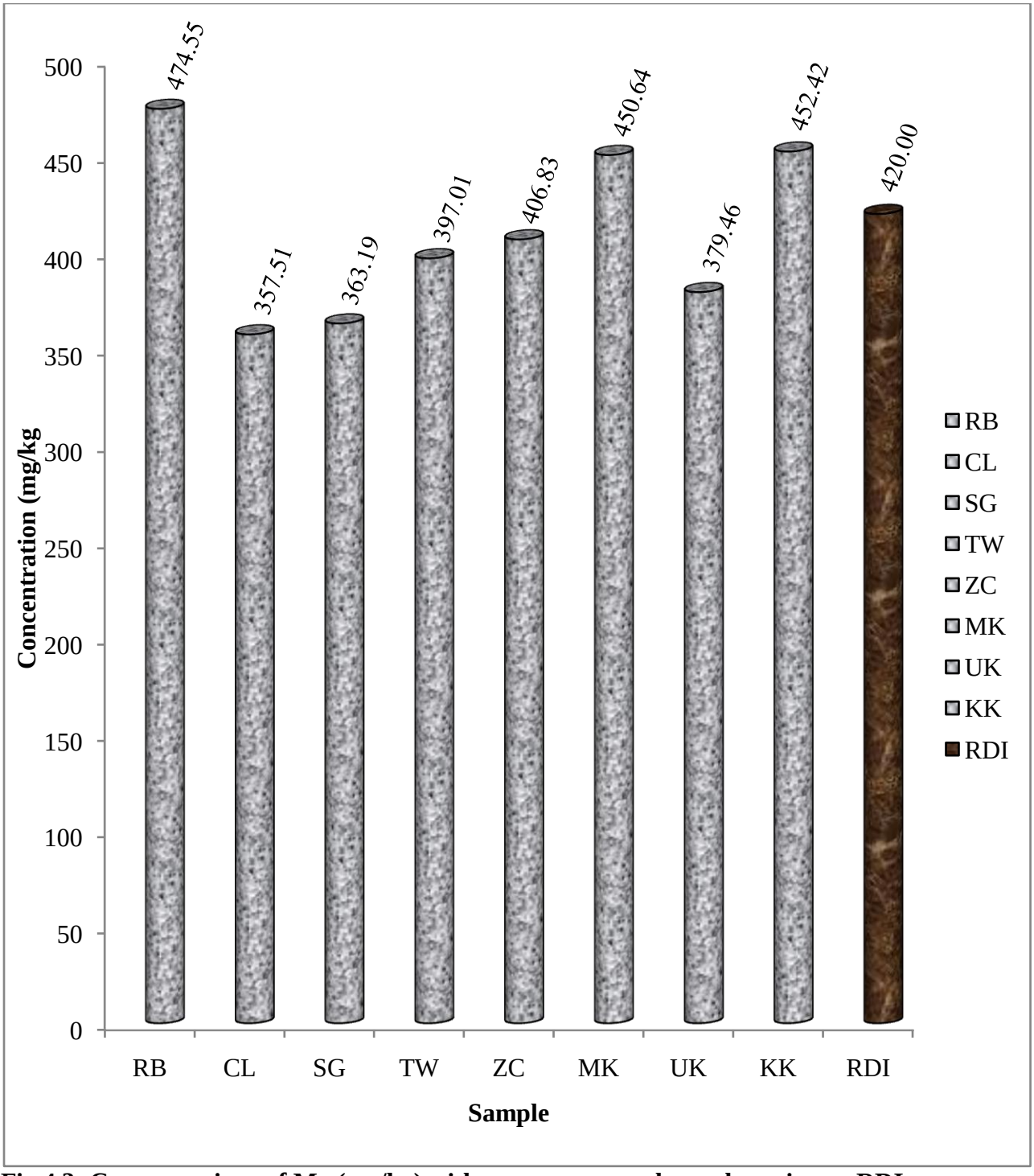


Fig 4.3: Concentrations of Mg (mg/kg) with respect to samples and maximum RDI

Key: RB = Dried beef, CL = Laboratory *Kilishi*, SG = Sabon Gari *Kilishi*, TW = Tudun Wada *Kilishi*, ZC = Zaria City *Kilishi*, MK = Malali Kaduna *Kilishi*, UK = Anguwan Sarki Kaduna *Kilishi*, KK = Kasuwa Kaduna *Kilishi* and RDI =.Recommended dietary intake.

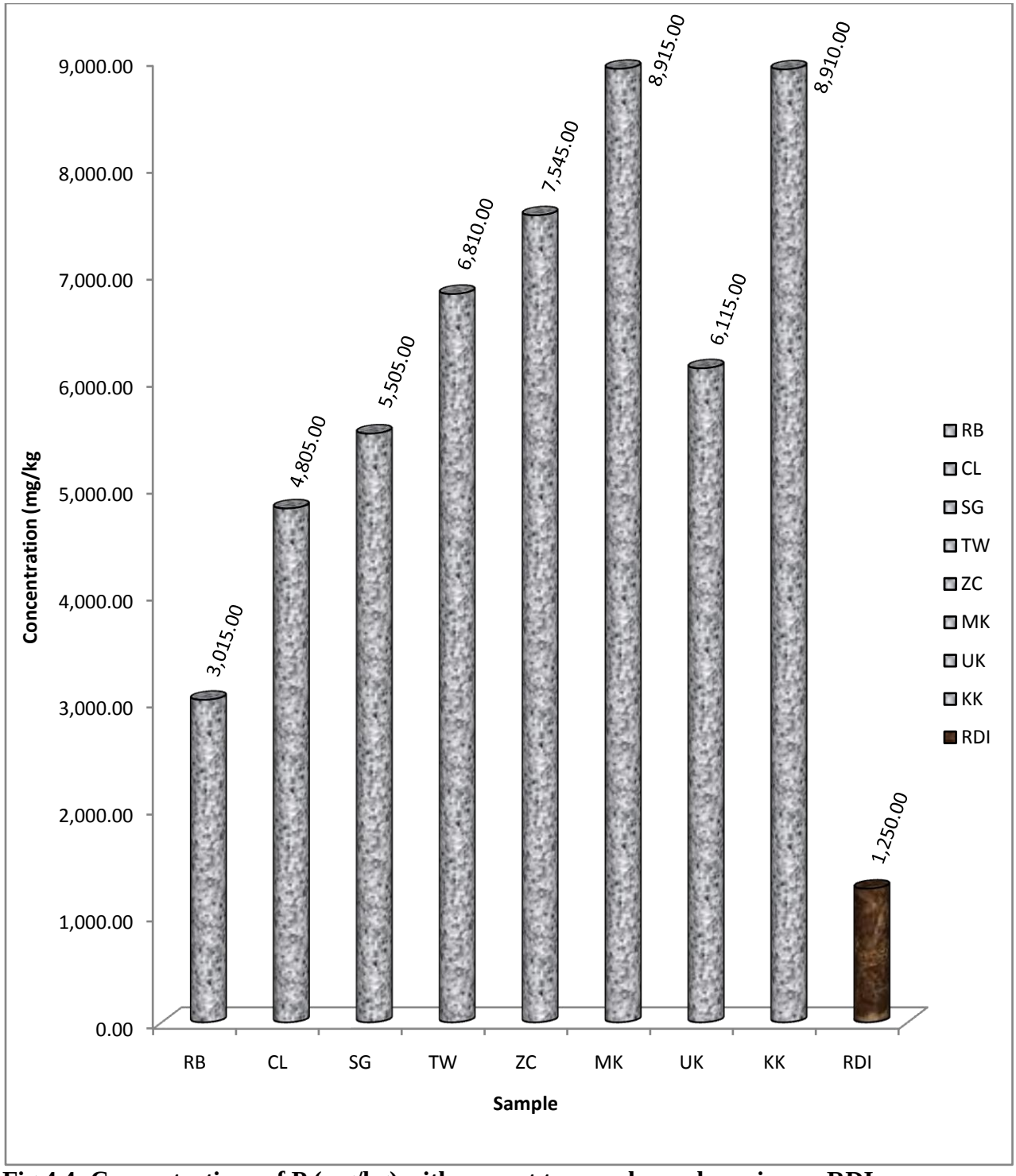


Fig 4.4: Concentrations of P (mg/kg) with respect to samples and maximum RDI

Key: RB = Dried beef, CL = Laboratory *Kilishi*, SG = Sabon Gari *Kilishi*, TW = Tudun Wada *Kilishi*, ZC = Zaria City *Kilishi*, MK = Malali Kaduna *Kilishi*, UK = Anguwan Sarki Kaduna *Kilishi*, KK = Kasuwa Kaduna *Kilishi* and RDI = Recommended dietary intake.

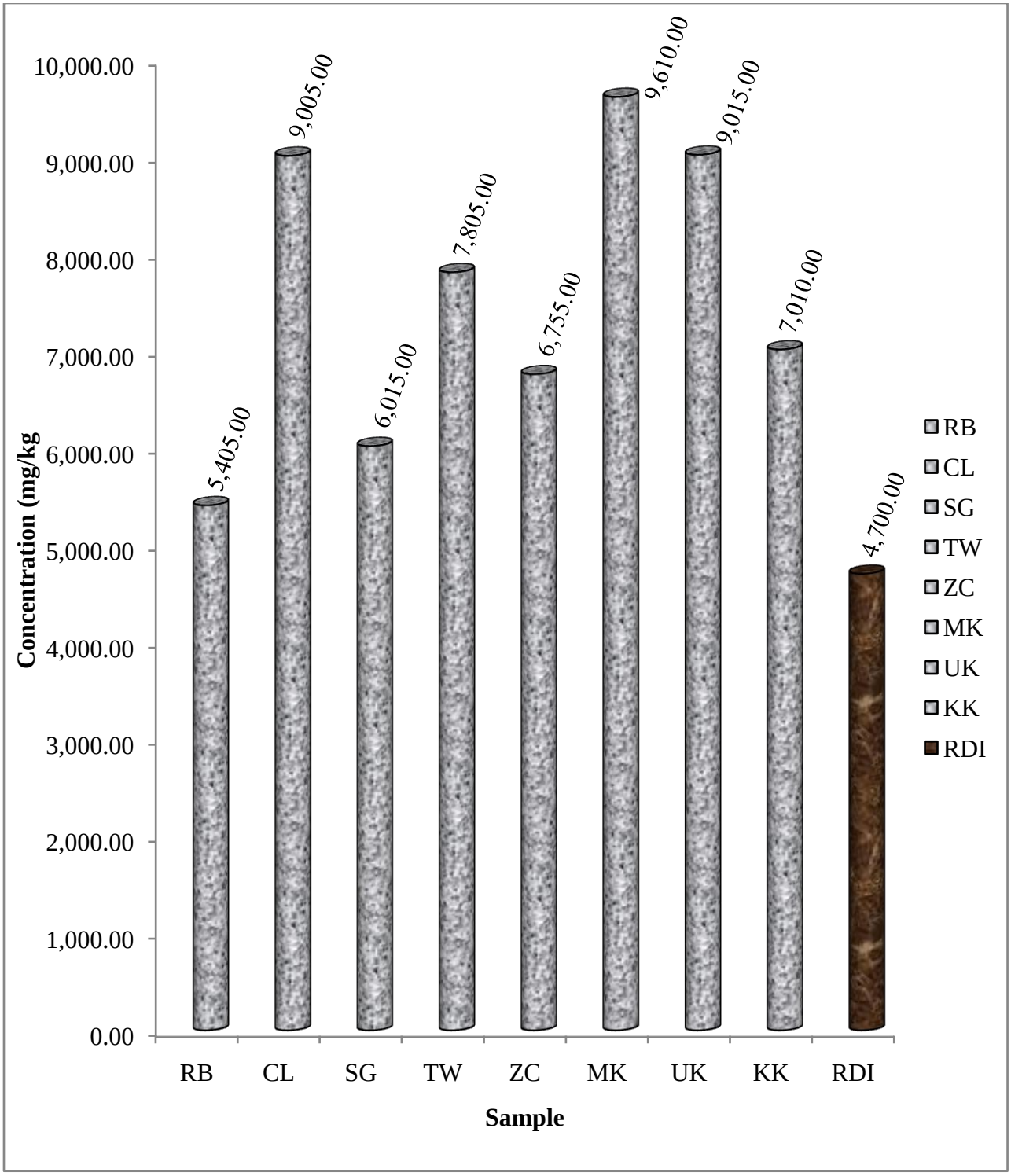


Fig 4.5: Concentrations of K (mg/kg) with respect to samples and maximum RDI

Key: RB = Dried beef, CL = Laboratory *Kilishi*, SG = Sabon Gari *Kilishi*, TW = Tudun Wada *Kilishi*, ZC = Zaria City *Kilishi*, MK = Malali Kaduna *Kilishi*, UK = Anguwan Sarki Kaduna *Kilishi*, KK = Kasuwa Kaduna *Kilishi* and RDI = Recommended dietary intake.

4.3 Heavy Metal Concentrations in Dried Beef and its *Kilishi* Samples

Table 4.2 below shows the mean and standard deviations of heavy metals. The highest value of Fe concentration of 192.80 $mgkg^{-1}$ was recorded in Tudun Wada *Kilishi* while the dried beef had the lowest value of 56.20 $mgkg^{-1}$. Copper concentration was highest in *Kilishi* from Sabon Gari of value 7.58 $mgkg^{-1}$, which is not significantly different from Anguwan Sarki sample ($p < 0.05$) while the lowest Cu value, 2.84 $mgkg^{-1}$ was obtained from dried beef. The concentration of Zn ranged from 78.51 to 135.43 $mgkg^{-1}$ with the highest value recorded in Sabon Gari *Kilishi* and lowest value in raw beef. Cobalt concentration was highest in *Kilishi* from Anguwan Sarki having a value of 0.68 $mgkg^{-1}$ while Co concentrations were not detected in Sabon Gari and Zaria City sample respectively. The value of cobalt in Laboratory *Kilishi* of 0.075 $mgkg^{-1}$ was not significantly different at $p < 0.05$ from the Tudun Wada sample. Manganese concentration was highest in ZC of value 19.50 $mgkg^{-1}$ and lowest in RB of value 1.32 $mgkg^{-1}$. The concentration of Pb ranged from 1.83 - 13.75 $mgkg^{-1}$, with the highest value in Zaria City and the lowest value in Tudun Wada samples respectively. There was no significant difference ($p < 0.05$) of Pb concentration from Laboratory, Sabon Gari, Kasuwa, Malali and Tudun Wada samples. The concentrations of cadmium (Cd) and chromium (Cr) were not detected respectively probably because they were below their detection limits. Nickel concentration was highest in Sabon Gari *Kilishi* (19.88 $mgkg^{-1}$) and lowest in Malali *Kilishi* (2.08 $mgkg^{-1}$) that was not significantly different from the value obtained in Laboratory sample ($p < 0.05$). The levels of Ni in dried beef and Tudun Wada *Kilishi* showed no significant differences ($p < 0.05$).

Table 4.2: Means and Standard Deviations of Heavy Metals Concentrations ($mgkg^{-1}$) in Dried Beef and its *Kilishi* Samples

S/C	Fe	Cu	Zn	Co	Mn	Pb	Cd	Cr	Ni
RB	56.20^{h}	2.84^{e}	78.51^{h}	0.03^{e}	1.32^{g}	8.98^{b}	BDL	BDL	2.68^{d}
CL	188.88^{b}	3.58^{d}	80.09^{g}	0.08^{d}	8.64^{f}	2.75^{cd}	BDL	BDL	2.08^{e}
SG	162.48^{d}	7.58^{a}	135.43^{a}	BDL	12.69^{b}	2.20^{d}	BDL	BDL	19.88^{a}
TW	192.80^{a}	4.48^{c}	130.28^{b}	0.08^{d}	12.24^{bc}	1.83^{de}	BDL	BDL	2.95^{d}
ZC	142.18^{g}	6.40^{b}	90.68^{e}	BDL	19.50^{a}	13.75^{a}	BDL	BDL	14.95^{bc}
MK	152.74^{e}	6.45^{b}	81.66^{f}	0.18^{c}	10.95^{d}	2.05^{d}	BDL	BDL	2.15^{e}
UK	148.88^{f}	7.10^{ab}	95.66^{d}	0.68^{a}	12.72^{b}	3.43^{c}	BDL	BDL	14.18^{c}
KK	167.58^{c}	4.58^{c}	100.89^{c}	0.40^{b}	10.38^{e}	2.10^{d}	BDL	BDL	15.43^{b}
M	151.46	5.37	99.15	0.18	11.06	4.63	BDL	BDL	9.28
CV	42.53	1.74	22.26	0.24	5.06	4.37	BDL	BDL	7.49
UL	80.00	8.00	50.00	0.16	11.00	0.50	0.50	0.10	0.50

Key: S/C = Sample code, RB = dried beef, CL = Laboratory *Kilishi*, SG = Sabon Gari *Kilishi*, TW = Tudun Wada *Kilishi*, ZC = Zaria City *Kilishi*, MK = Malali Kaduna *Kilishi*, UK = Anguwan Sarki Kaduna *Kilishi*, KK = Kasuwa Kaduna *Kilishi*, BDL = Below detection limit, M = total mean, CV = coefficient variance, SE = standard error of means, UL = tolerable upper intake level ($mgkg^{-1}$).
a-f: means along the same column with different superscript are significantly different ($P < 0.05$)

4.4 Microbial Count in Dried Beef and its *Kilishi* Samples

The results obtained of Mesophillic aerobic plate count, *Staphylococcus* count, Coliform count and fungi count are presented in Table 4.3. This showed that the dried beef and its *Kilishi* samples were contaminated with microorganisms despite the dried and hard texture of the samples. Total aerobic plate counts was highest in Tudun Wada *Kilishi* (9.03×10^5 cfu/g) that had no significant difference at $p < 0.05$ with value (9.01×10^5 cfu/g) obtained from Anguwan Sarki *Kilishi*, while aerobic plate counts were below detection limit in dried beef and Laboratory *Kilishi* samples. Aerobic plate counts values in samples from Kasuwa, Sabon Gari, Malali and Zaria City were significantly different ($p < 0.05$). *Staphylococcus* counts ranged from 1.56×10^5 to 3.62×10^7 cfu/g, with the highest counts value from Anguwan Sarki sample and the lowest from Laboratory sample. The *Staphylococcus* counts from Kasuwa, Tudun Wada, Sabon Gari, Zaria City and Malali *Kilishi*, and from the dried beef were significantly different ($p < 0.05$). Coliform was highest in Tudun Wada *Kilishi* with a count of 9.61×10^6 cfu/g, while no significant growth was determined below the detection limit in dried beef. Coliform counts of 0.11×10^2 cfu/g in *Kilishi* from Laboratory had significantly low value than the values obtained in other *Kilishi* samples. The results showed that fungi counts was highest in *Kilishi* from Kasuwa (2.02×10^5 cfu/g) that had no significant difference at $p < 0.05$ to values obtained from Anguwan Sarki, Tudun Wada and Sabon Gari samples. The lowest fungi counts value was obtained from the Laboratory *Kilishi* (1.01×10^4 cfu/g), while its value in dried beef sample was found to be 3.01×10^4 cfu/g.

Table 4.3: Means and Standard Deviations of the Microbial Counts (cfu/g) of Dried Beef and its *Kilishi* Samples

Sample Code	Aerobic Mesophillic count	Staphylococcus count	Coliform count	Fungi count
RB	ND^{f}	$4.21 \times 10^{6} \pm 0.14^{g}$	ND^{h}	$3.01 \times 10^{4} \pm 0.00^{c}$
CL	ND^{f}	$1.56 \times 10^{5} \pm 0.01^{h}$	$0.11 \times 10^{2} \pm 0.00^{g}$	$1.01 \times 10^{4} \pm 0.00^{d}$
SG	$4.01 \times 10^{5} \pm 0.01^{c}$	$2.41 \times 10^{7} \pm 1.41^{d}$	$1.30 \times 10^{2} \pm 0.07^{f}$	$2.00 \times 10^{5} \pm 0.00^{a}$
TW	$9.03 \times 10^{5} \pm 0.01^{a}$	$3.22 \times 10^{7} \pm 0.71^{c}$	$9.61 \times 10^{6} \pm 0.07^{a}$	$2.01 \times 10^{5} \pm 0.01^{a}$
ZC	$2.01 \times 10^{5} \pm 0.01^{e}$	$1.93 \times 10^{7} \pm 0.71^{e}$	$5.31 \times 10^{6} \pm 0.07^{b}$	$1.01 \times 10^{5} \pm 0.01^{b}$
MK	$3.01 \times 10^{5} \pm 0.07^{d}$	$1.52 \times 10^{7} \pm 0.00^{f}$	$4.21 \times 10^{6} \pm 0.07^{c}$	$1.01 \times 10^{5} \pm 0.01^{b}$
UK	$9.01 \times 10^{5} \pm 0.01^{a}$	$3.62 \times 10^{7} \pm 0.71^{a}$	$6.01 \times 10^{5} \pm 0.01^{e}$	$2.01 \times 10^{5} \pm 0.01^{a}$
KK	$7.00 \times 10^{5} \pm 0.00^{b}$	$3.31 \times 10^{7} \pm 1.41^{b}$	$1.12 \times 10^{6} \pm 0.71^{d}$	$2.02 \times 10^{5} \pm 0.01^{a}$
MEAN	4.26×10^{5}	2.05×10^{7}	4.29×10^{6}	1.31×10^{5}
CV	0.21	0.30	0.61	0.50
SE	0.01	0.61	0.26	0.01
PL	1×10^{6}	1×10^{6}	1×10^{2}	1×10^{2}

Key: RB = dried beef, CL = Laboratory *Kilishi*, SG = Sabon Gari *Kilishi*, TW = Tudun Wada *Kilishi*, ZC = Zaria City *Kilishi*, MK = Malali Kaduna *Kilishi*, UK = Anguwan Sarki Kaduna *Kilishi*, KK = Kasuwa Kaduna *Kilishi*, ND = not detected at lower concentration; CV = coefficient variance; SE = standard error of means, PL = permissible limit.

a-f: means along the same column with different superscript are significantly different ($P < 0.05$).

4.5 Microorganisms Isolates in Dried Beef and its *Kilishi* Samples

Table 4.4 below showed the isolated varieties of microorganisms that contaminated the studied samples. The isolated bacteria species belonged to the *Enterobacteriaceae* family of *Salmonella* spp.*, Staphylococcus aureus, Escherichia coli, Klebsiella* spp. and *Bacillus* spp.. *Salmonella* spp. were detected in *Kilishi* samples from Malali, Anguwan Sarki and Kasuwa. *Staphylococcus aureus* were isolated in all the samples. *E. coli* were isolated in *Kilishi* from Tudun Wada, Zaria City, Malali, Anguwan Sarki and Kasuwa. *Bacillus* spp. were detected in dried beef and *Kilishi* samples from Laboratory, Sabon Gari, Tudun Wada and Zaria City, while *Klebsiella* spp. were isolated in *Kilishi* from Tudun Wada, Zaria City, Malali, Anguwan Sarki and Kasuwa. The isolated fungi were of the genus *Aspergillus* spp., *Penicillium* spp. *and Geotrichum candidum*. *Aspergillus* spp. were isolated in dried beef and *Kilishi* samples from Sabon Gari, Tudun Wada, Anguwan Sarki and Kasuwa. *Penicillium* spp. were equally detected in dried beef and *Kilishi* samples from the Laboratory, Sabon Gari, Anguwan Sarki and Kasuwa. *Geotrichum candidum* were isolated in Malali, Anguwan Sarki and Kasuwa *Kilishi* samples.

Table 4.4: Genera of Bacteria and Fungi Isolated from Dried Beef and its *Kilishi* Samples

Sample Code	Organisms isolated
RB	*Staphylococcus aureus, Bacillus* spp., *Penicillum* spp., *Aspergillus* spp.
CL	*Staphylococcus aureus, Bacillus* spp., *Penicillum* spp.
SG	*Staphylococcus aureus, Bacillus* spp., *Penicillum* spp., *Aspergillus* spp.
TW	*Escherichia coli, Klebsiella* spp., *Staphylococcus aureus, Bacillus* spp., *Aspergillus* spp.
ZC	*Escherichia coli, Klebsiella* spp., *Staphylococcus aureus, Bacillus* spp., *Geotricum candidum*
MK	*Escherichia coli, Salmonella* spp., *Klebsiella* spp., *Staphylococcus aureus, Bacillus* spp., *Geotricum candidum*
UK	*Salmonella* spp., *Klebsiella* spp., *Escherichia coli, Staphylococcus aureus, Penicillum* spp., *Aspergillus* spp., *Geotrichum candidum*
KK	*Salmonella* spp., *Klebsiella* spp., *Escherichia coli, Staphylococcus aureus, Penicillum* spp., *Aspergillus* spp., *Geotrichum candidum*

Key: RB = Dried beef, CL = Laboratory *Kilishi*, SG = Sabon Gari *Kilishi*, TW = Tudun Wada *Kilishi*, ZC = Zaria City *Kilishi*, MK = Malali Kaduna *Kilishi*, UK = Anguwan Sarki Kaduna *Kilishi*, KK = Kasuwa Kaduna *Kilishi*, spp. = species.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Proximate Compositions in Dried Beef and its *Kilishi* Samples

5.1.1 Moisture content

From the results in Table 4.1, it can be seen that percent moisture content ranged between 6.46 - 9.23%. According to Ingram and Simonsen (1980), meat with moisture content of 20 and 15% has the ability of inhibiting some bacteria and fungi species respectively. Following their opinion, it therefore implies that the studied samples can inhibit the growth of microbes (lower moisture content). Similarly, this result agreed with moisture content values in *Kilishi* samples (7.50% and 8.67 - 10.00%) reported by Igene *et al*. (1990) and Olusola *et al.* (2012) respectively. However, Isah and Okubanjo (2012) reported higher values of moisture content than the current results of 16.65% and between 19.75 and 23.30% in dried meat and *Kilishi* samples respectively. The higher moisture content in *Kilishi* samples could be attributed to the step-wise drying in their processing technique (Mbofung, 1993). It could also be as a result of the environmental conditions and the infusion ingredients paste used during its production.

5.1.2 Crude fat

From Table 4.1 it can be seen that crude fat was found to range from 17.07 to 22.42%. In comparison with Igene (1988) and Jones *et al.* (2001) who discovered that *Kilishi* is very high in fat content (of not less than 25%), the results of this study showed that the there generally appeared to be more seperable fat in the trimmed raw beef before its production to Kilishi. The results obtained were in agreement with the

percent fat in *Kilishi* (17.80% - 23.23%) reported by Igene *et al.* (1990). However, Mgbemere *et al.* (2011) reported lower values of percent fat than the current study in *Kilishi* (13.4) and Adebayo-Tayo (2011) reported in *Banda* dried beef (1.86 - 6.21%). The high values noticed in *Kilishi* samples as compared to the low value obtained in raw beef could also be attributed to the lowest moisture and fat content relationship, due to the fact that they relate inversely (Fakolade, 2012).

5.1.3 Crude protein

From Table 4.1, it can be seen that the crude protein values ranged from 52.92 to 67.94%. The result of this study agreed with the values of crude protein of 54.20% in *Banda* beef products reported by Oladejo and Adebayo-Tayo (2011) and 59.70 to 62.30% in *Kilishi* reported by Olusola *et al.* (2012). However, Rasairo *et al.* (1994), and Egbunike and Okubanjo (1999) reported higher values of protein content than this result of ranged 68.1 to 71.8% in *Kilishi* and 56.7 to 72.3% in beef cacina respectively. Igene *et al*. (1993) and Mgbemere *et al.* (2011) reported lower percent of protein than the current study of 50.0 and 49.0% in *Kilishi* samples respectively. The difference in protein content observed between *Kilishi* samples may be attributed to their trimmed connective tissues of the raw beef, the groundnut cake and other ingredients mixed in their respective production.

5.1.4 Ash content

Table 4.1 shows the ash content that ranged between 6.81 and 9.42%, a range that is similar to the results in *Kilishi* samples of 6.72% and 7.80 - 9.60% reported by Igene *et al.* (1990) and Jones *et al.* (2001) respectively. Similarly, Ogunsola and Omojola (2008) reported ash content value of 8.78% and Idowu *et al.* (2010) reported ash content value of 7.1% in *Kilishi* samples respectively. However, Faleye and

Fagbohun (2012) reported lower ash contents than this study of range 2.05 to 3.07 g100g^{-g} in *Tinco* beef products. The relatively high values of percentage ash content in *Kilishi* samples could be due to the condiments used during their preparations. It may also be due to the resultant dirt on the meat pieces during sun drying in the opened places or due to adhering dirt on the roasting medium. According to researchers, ash content is a measure of the amount of minerals in meat samples (Ogunsola and Omojola, 2008; Faleye and Fagbohun, 2012).

5.1.5 Crude fibre

As shown in Table 4.1, the results of fibre content in this study ranged from 1.26 to 3.42%. Francis (2007) postulated that low fibre content of 2.5% would help in easy digestion of the protein present in meat. Following his opinion, it therefore implies that with the exception of *Kilishi* from Tudun Wada with 3.42% crude fibre, the studied *Kilishi* samples are good in dietary fibre that promotes easy digestion of high protein. The result of this study agreed with the fibre content in *Kilishi* (2.96 - 4.12%) reported by Olusola *et al.* (2012). However, Igene *et al.* (1993) reported higher value than the current result of 9.8% in *Kilishi*, while Faleye and Fagbohun (2012) reported lower values of crude fibre than the current result range from 1.09 to 1.28 g100g^{-1} in *Tinco* beef products. Determination of crude fibre in dried beef may be due to the inability of the trimmed fresh beef to remove excess connective tissues, dirty spots and any visible contamination. The relatively high values of fibre contents in *Kilishi* probably was due to the rate of absorption and adsorption of the dried raw meat slices in the different spices mixture in *Kilishi* production. These spices are of plant origin, hence are likely to be fibrous (Brummer and Cui, 2005; Olusola *et al.*, 2012).

5.1.6 Carbohydrate content

Results in Table 4.1 shows carbohydrate content values (1.72 - 8.32%) of the dried beef and its *Kilishi* samples varied significantly ($p < 0.05$). According to Idowu *et al.* (2010), meats with low carbohydrate content value (8.05%), make a good source of low dietary carbohydrate to diabetic patients. Following this opinion, it therefore implies that the studied *Kilishi* samples can be good diet for diabetic petients. Similarly, the result of this study agreed with the carbohydrate content of 0.06 and 7.35% in dried beef and *Kilishi* samples respectively (Olusola *et al.*, 2012). However, Mgbemere *et al.* (2011) reported higher values of carbohydrate (14.8 and 18.9%) in *Kilishi* samples, while Faleye and Fagbohun (2012) reported lower value (4.48%) in *Tinco* beef product. High values of Carbohydrate contents in *Kilishi* samples are a reflection of the nature of the components that constituted the spices used in their samples production (Ogunsola and Omojola, 2008).

5.2 Mineral Contents in Dried Beef and its *Kilishi* Samples

5.2.1 Sodium (Na)

As seen in Figure 4.1, the results of Na content ranged between 785.00 and 8,281.50 $mgkg^{-1}$. This result is not in conformity with the lower Na content of 180.06 ppm in beef reported by Goncu *et al.* (2008) and 29.00 to 33.55 $mg100g^{-1}$ in *Tinco* beef product reported by Faleye and Fagbohun (2012). Higher concentrations of Na may be attributed to the selected breeding feed of the slaughtered cow used for the analysis. The higher values noticed in *Kilishi* samples are also most likely due to salt and spices proportions added during the processing. The recommended daily intake of Na is 1500 - 2400 mg/day (Institute of Medicine, 2002).

5.2.2 Calcium (Ca)

Figure 4.2 showed that Ca contents ranged from 337.51 to 459.55 $mgkg^{-1}$. The results obtained from this study for Ca is similar to those recorded in *Kilishi* meats by Ogbonnaya and Linus (2009) and Jones *et al.* (2001) who carried out analysis of Kilishi and obtained values of 55.69 and 54.69 $mg100g^{-1}$ respectively. However, Faleye and Fagbohun (2012) recorded lower Ca values than the current result in *Tinco* beef products that ranges from 20.66 to 24.10 $mg100g^{-1}$. The lower Ca values obtained in *Kilishi* samples as compared to the higher value obtained in dried beef could be attributed to the lost in moisture content that would lead to an increase in mineral content per gram (Fakolade, 2012). The significantly different in values ($P < 0.05$) noticed in *Kilishi* samples are also most likely due to the ingredients used in their respective production. Recommended daily dietary intake of Ca is 800 - 1,300 mg/day (Institude of Medicine, 2002).

5.2.3 Magnesium (Mg)

The Mg content of this study (Figure 4.3) ranged from 357.51 to 474.55 $mgkg^{-1}$. Ogbonnaya and Linus (2009) reported higher Mg contents in *Kilishi* than the present results of value 114.97 $mg100g^{-1}$. In the same vein, Zahurul *et al.* (2011) reported Mg contents ranged between 27.88 and 1,372.54 $mgkg^{-1}$ in beef. However, Jones *et al.* (2001) recorded lower Mg value in *Kilishi* than the present results of 48.54 ppm. The decreased in concentrations of Mg in *Kilishi* samples as compared to the high value obtained in dried beef may be due to moisture lost that would indeed lead to an increased in mineral content per gram (Fakolade, 2012). The relatively high values of Mg content in *Kilishi* samples could be due to the ingredients proportions

during processing. The recommended daily dietary intake of Mg by humans is 240 to 420 mg/day (Institute of Medicine, 2002).

5.2.4 Phosphorus (P)

As presented in Figure 4.4, the concentrations of phosphorus in the study samples ranged from 3,015.00 to 8,915.00 $mgkg^{-1}$. The P contents obtained from this study were higher than the P values of 240.30 ppm reported by Goncu *et al.* (2008) in beef meats and 392.42 $mg100g^{-1}$ reported by Ogbonnaya and Linus (2009) in *Kilishi*. High values of P recorded could be because of the high protein content in the beef and the ingredients used in their preparations especially garlic (Ogbonnaya and Linus, 2009; Shereen, 2015). According to FAO/WHO (2001), the recommended daily intake level of P is 700 to 1,250 mg/day.

5.2.5 Potassium (K)

Figure 4.5 depicts the K results obtained, 5,405.00 to 9,610.00 $mgkg^{-1}$. Higher values of K recorded may be attributed to the kind and degree of the feed intake of the cattle that was slaughtered (Baykov *et al.*, 1996). However, Goncu *et al.*, 2008, and Faleye and Fagbohun, 2012 recorded lower values of K compared to the current study in beaf and beef product known as *Tinco* of 515.03 ppm and 47.39 $mg100g^{-1}$ respectively. Higher values of K in the *Kilishi* samples as compared to lower value in dried beef could be because of the groundnut used in their production. According to Institude of Medicine (2002), the daily intake of K is 4,700.00 mg/day.

5.3 Heavy Metals Concentrations in Dried Beef and its *Kilishi* Samples

5.3.1 Iron (Fe)

The levels of Fe contents in the study samples (Table 4.2) ranged between 56.20 and 192.80 $mgkg^{-1}$. In comparison with the permissible limit of 80.00 $mgkg^{-1}$ recommended by Institute of Medicine (2003) all *Kilishi* samples had Fe concentrations above permisible limit. Therefore, these samples could be used as a better source of Fe element (Ponka *et al.*, 2007). The higher values of Fe thus recorded may be attributed to the age, physiological state and feed intake of the slaughtered cattle (Sabir et al. 2003). Similarly, high values of Fe recorded in the *Kilishi* samples could be because of the ingredients used in their preparations. The levels of Fe in the studied samples were in concordant with the value of 198.55 $mgkg^{-1}$ obtained in beef by Zahurul *et al.* (2011). However, Syed *et al*. (2003) recorded Fe values that ranged between 2,390 and 2,415 $mgkg^{-1}$ in beef, which values are by far higher than the current results. This study also gave higher values of Fe compared to those reported by Bendeddouche *et al.* (2014) in beef of ranged 68.72 to 84.22 μgg^{-1}.

5.3.2 Copper (Cu)

As seen in Table 4.2, results of Cu levels ranged between 2.84 and 7.58 $mgkg^{-1}$. Although Cu is an essential element, it is toxic above threshold level and the maximum limit intake was set from 1 to 8 mg/day (WHO, 1996; Stern, 2010). Our data about Cu in all the samples had Cu concentrations below the tolerable upper intake. The results of this study were similar to the Cu concentrations ranged between 1.44 and 6.92 ppm in meat products reported by Dalia and Bassa (2015). However, Zahurul *et al.* (2011) and Amani and Lamia (2012) reported higher Cu values in meat products than these results that ranged from 0.75 to 165.02 $mgkg^{-1}$ and 11.11 to 18.51

and mgkg^{-1} respectively. Lower values of Cu obtained in this study could be attributed to the age, physiological state and feed intake of the slaughtered cow (Baykov et al., 1996). It could also be due to the processing of *Kilishi* that exposed the raw beef to traffic contaminants, dust as well as copper oxide from the welding operations that were closed to some *Kilishi* production spots.

5.3.3 Zinc (Zn)

The obtained results in Table 4.2 show that Zn concentrations of the study samples ranged from 78.51 to 135.43 mgkg^{-1}. The levels of zinc found in the present study are similar to those of Amani and Lamia (2012) who recorded Zn levels of range 16.74 to 147.82 µgg^{-1} in meat products. All the values in the studied samples were above the permissible limit (50 mgkg^{-1}) set by the Australia- New Zealand Food Authority (ANZFA, 2001). Therefore, these samples could be use as a better source of Zn element (Okubanjo, 1997). However, Zahurul *et al.* (2011) reported higher mena value of Zn (200.20 mgkg^{-1}) in meat products than results of the current study. High values of Zn could be attributed to the age, physiological state and feed intake as well as on living conditions of the slaughtered cow (Baykov et al., 1996). It could also be attributed to exposure of the raw beef to Zn pollutants in dust and traffic contaminants during its processing as well as the ingredients used (Kathleen, 2003; ATSDR, 2004).

5.3.4 Cobalt (Co)

The concentrations of Co in this study (Table 4.2), ranged between 0.00 and 0.68 mgkg^{-1}. From these results, it can be seen that the concentrations of Co in dried beef and *Kilishi* samples from the Laboratory, Sabon Gari and Zaria City were lower than the permissible limit of 0.16 mgkg^{-1} set by ATSDR (2002), while Co concentrations in *Kilishi* from Malali, Anguwan Sarki and Kasuwa were higher than

the permissible limit. The values measured in this study were lower compare to the Co values of 4.0 and 1.0 $mgkg^{-1}$ recorded in local and Indian cow meats respectively (Zahurul *et al.*, 2011). High Co values could be due to contamination of the raw beef through the deposition of Co contaminants from vehicle emission and from the dirty slaughter places. It could also arise from contaminated poultry feeds and processing of the raw beef.

5.3.5 Manganese (Mn)

The results of Mn concentrations in the study samples (Table 4.2), ranged between 1.32 and 19.50 $mgkg^{-1}$. Upper tolerable intake of Mn is 11 mg/day (Institute of Medicine, 2003). From these results, it can be seen that *Kilishi* from thee Laboratory, Malali and Kasuwa had Mn levels below the upper tolerable intake for humans, while the levels of Mn in *Kilishi* from Sabon Gari, Tudun Wada, Zaria City and Anguwan Sarki were above tolerable intake and thus, may be detrimental to consumers after long accumulation (Narayanan, 2007). However, these results agreed with the findings of some authors (Demirezen and Uruc, 2006; Amani and Lamia, 2012), who reported Mn levels in meat products that ranged from 3.98 to 10.00 $\mu g100g^{-1}$, and 11.79 to 32.67 μgg^{-1} respectively. Syed *et al.* (2003) had reported lower values of Mn in beef than the results of the current study of ranged between 1.00 and 3.00 $mgkg^{-1}$. Higher Mn values may be attributed to the contamination of raw beef that arises from contaminated poultry feeds and drinking water source of the slaughtered cow, and its processing. It may also be attributed to the fact that Kilishi production spots were located closed to the roadside and welding shop, which toxic form of Mn is reported to come from gasoline and metallurgy pollutants (Obasoham, 2008; Wilson, 2014).

5.3.6 Lead (Pb)

Lead concentrations in this study (Table 4.2), varied between 1.83 and 13.75 $mgkg^{-1}$ and were above the recommended threshold level of 0.5 $mgkg^{-1}$ (FAO/WHO, 2001). This indicated that these samples might be detrimental to health of its consumers after long accumulation, with respect to Pb. However, these results agreed with the Pb values in beef samples of 3.00 $mgkg^{-1}$ and 8.80 µg/g reported by Syed *et al*. (2003) and Amani *et al.* (2012) respectively. Zahurul *et al.* (2008) reported higher values of Pb (24.9 and 43.37 $mgkg^{-1}$) than the present results in local and Indian cow meats respectively. Excessive amount of Pb could not be attributed to deposition of contaminants from vehicle and welding generator's emissions during raw beef processing alone. It could emanate mainly from grazing of the slaughtered cow on contaminated soil, and feeding on contaminated feeds and water sources (Sabir *et al.*, 2003). Moreso, Pb is the most prevalent heavy metal contaminant in the opened environment of *Kilishi* processing (Benneth, 1981). The spices used during the processing of *Kilishi* products could attribute to their high Pb concentrations (Badau *et al.*, 2013).

5.3.7 Cadmium (Cd)

Table 4.2 shows that Cd was not detected in any sample. The non-detection of Cd in this study could be probably due to Cd was below its detection limit. It could also be due to absent of Cd metal in the slaughtered cow's feed used for the analysis (Damerau *et al.*, 2012). Processing of these Kilishi also increased the chances of its contamination with Cd pollutants from open air. However, Zahurul *et al.* (2011) and Bendeddouche *et al.* (2014) reported Cd in beef samples of 8.04 $mgkg^{-1}$ and ranged

1.56 to 1.71 μgg^{-1} respectively. According to EC (2008) Cd intake limits is 0.05mg/kg/day.

5.3.8 Chromium (Cr)

In Table 4.2, it can be seen that the results of Cr concentrations were not detected in all the samples. Probably, this might be as a result Cr was below the detection limit. Contamination of the slaughtered cow's feed by toxic metals including Cr, cannot be entirely avoided given the prevalence of these pollutants in the opened environment of the *Kilishi* processing, which could lead to an airborne exposure to hexavalent Cr (Kithiia, 2006 and Narayanan, 2007). This result is not in agreement with Cr values in beef samples of 0.23 - 1.22 μgg^{-1} and 1.22 mgkg^{-1} reported by Akam *et al.* (2010) and Zahurul *et al.* (2011) respectively. An adequate Cr intake is 0.1 and μg/day (Rose *et al.*, 2010).

5.3.9 Nickel (Ni)

Table 4.2 shows the concentrations of Ni that ranged from 2.08 to 19.88 mgkg^{-1}. According to Institute of Medicine (2003), the upper tolerable intake level of nickel for children (1–3 years old) and adults (19–70 years old) is 7 and 50 μg/day respectively. The levels of Ni found in these samples exceeded the upper tolerable intake levels of this element. This indicated that the study samples could caused Ni toxicity after long time of its consumption by humans. However, the results obtained from this study for Ni are similar to those values that ranged from 8.20 to 24.00 μgg^{-1} recorded by Demirezen and Uruc (2006) in meat products. However, Zahurul *et al.* (2011) had reported lower Ni values than the values of the current study of ranged 0.00 to 4.85 mgkg^{-1} in meat products. The significantly high concentration of Ni in Sabon Gari sample could be from welder's generators exhausts obtained from the

petroleum and from the dumb wastes which its production spot was closely located alone (Syed *et al.*, 2003; Narayanan, 2007; Obasohan, 2008). Contamination could also be attributed to raw beef, through direct exposure to Ni found in the dust and air during processing Mwegoha and Kihampa (2010).

5.4 Microbiological Counts in Dried Beef and its *Kilishi* Samples

5.4.1 Mesophillic aerobic plate counts

From the results (Table 4.3), the levels of aerobic plate counts ranged from zero to 9.03 x 10^5 cfu/g. According to Pearson (1986), aerobic plate count should not exceed 2.5 x 10^5 to 1.0 x 10^8 cfu/g. Thus, these results showed lower values than the suggested value 2.5 x 10^5 to 1.0 x 10^8 cfu/g, which implies that the study samples are free from microbes that enhanced their spoilage. The results obtained were similar to the values recorded by by Adzitey *et al.* (2011) and Odey *et al.* (2013) in *Kilishi* and beef ranging from 3.73 to 1.67 x 10^5 and 1.4 to 2.3 x 10^5 cfu/g respectively. However, Adetunde *et al.* (2011) reported higher values of viable bacteria count (2.12 - 3.26 x 10^7 and 4.16 - 9.97 x 10^7 cfu/g) in beef meats from Ghana than the present study, while Okonko *et al.* (2013) reported lower bacteria count (2.1 x 10^2 to 4.32 x 10^3 cfu/g) in *Kilishi*. The non-detection of *Mesophillic* counts in dried beef and *Kilishi* prepared from Laboratory may be probably due to its detection limit or proper sanitary of the Laboratory. The presence of aerobi plate counts in *Kilishi* from commercial spots may be due to its contamination from the roadside dust, flies or some moisture contents that might cause the growth of some microorganisms.

5.4.2 *Staphylococcus* counts

In the present study (Table 4.3), values of *Staphylococcus* counts of ranged 1.56 x 10^5 - 3.62 x 10^7 cfu/g exceeded the *Staphylococcus* counts permissible limit of 10^2 cfu/g recommended by FAO/WHO (2001). This indicates poor sanitary quality of the samples, which may pose health risks to human in respect to *Staphylococcus*. This result is not in agreement with the previous findings (Laciokova *et al.*, 2004; Fonkem *et al.*, 2010) who reported lower values of *Staphylococcus* counts in *Kilishi* and meat products of values 2.80 × 10^4 and 1.43 × 10^2 cfu/g respectively. High values of *Staphylococcus* count contamination in the samples might affirm the cross contamination through processing as reported by Norrung *et al.* (2009). The salt content in the samples might also permit the growth of *Staphylococcus aureus* as reported by Odey *et al.* (2013).

5.4.3 Coliform counts

In Comparison to permissible Coliform consumption limit of 10^2 cfu/g recommended by FAO/WHO (2001), it could be seen in Table 4.3 that samples prepared from the Laboratory and Sabon Gari were within the permissible ranged, indicating the hygienic properties of the samples. Coliform values obtained in samples from Tudun Wada, Zaria City, Malali, Anguwan Sarki and Kasuwa exceeded the permissible limit (10^2 cfu/g), indicating poor sanitation and problems with the process control and handling of the raw beef and their *Kilishi* samples (Solberg *et al.*, 1976). However, this result is in agreement with the results of studies done by Odey *et al.* (2013) who reported Coliform counts ranged from 1.0 to 3.5 x 10^5 cfu/g in meat and meat products. In the contrary, Adetunde *et al.* (2011) reported lower total Coliform counts in beef (1.6 x 10^3 cfu/g) than the current results. The non-detection of

Coliforms in dried beef may be probably due to its detection limit or non-app.lication of water on it. High values of total Coliforms in the *Kilishi* samples might occur because of raw beef contact with surfaces that had been contaminated from fecal matter. It could also be from the used of contaminated water for the preparation of the slurry, used of unsterilized knives and improper handling of the beef meats.

5.4.4 Fungi (moulds and yeasts) counts

Table 4.3 shows fungi counts ranged from 1.0 x $l0^4$ to 2.0 x 10^5 cfu/g. Results of this study indicate that the levels of fungi growth in all the samples were significantly higher than 1.0 x 10^2 cfu/g permissible limits (FAO/WHO, 2001). However, Fonkem *et al.* (2010), prosposed that the levels of fungi counts has to be higher than the values of the aerobic plate counts of 1.5 x $l0^5$ to 1.0 x 10^8 cfu/g suggested by Pearson (1986), before the sample could be detrimental to fungi effect. Following this opinion, it therefore implies that the studied samples did not contribute to an outbreak of food-borne illness that might be caused by fungi contamination (Norrung *et al.*, 2009). Studies done in meat products by Berghoef *et al.* (2003) and Aydin *et al.* (2009) showed similar results with the present study of fungi counts of 10^5 and 10^2 cfu/g respectively. However, Pinho and Furlong (2000) reported lower fungi counts values in *Pizza* beef products of range between 1.3 x 102 and 2.9 x 10^2 cfu/g. High fungi counts values in *Kilishi* samples could be attributed to exposure of the raw beef to dust and dirty environment during its processing.

5.5 Microorganisms Isolates in Dried Beef and its *Kilishi* Samples

The genera of bacteria detected in this study sample as presented in Table 4.4 are disease-causing organisms (James *et al.*, 2000). *Salmonella* spp., which is not to be present in food samples according to World Food Programme (2012), was isolated

in *Kilishi* samples from Malali, Anguwan Sarki and Kasuwa. This therefore implies that regular consumption of these samples may cause food poisoning (WFP, 2012). The prevalence of *Salmonella* in these samples could attribute to the use of contaminated waters in their processing.

Staphylococcus aureus were isolated in all the study samples, and this indicates that these samples can lead to *Staph* infections in humans that consume these samples regularly (Bacteriological Analytical Manual, 1992). *Escherichia coli* were isolated in *Kilishi* samples from Tudun Wada, Zaria City, Malali, Anguwan Sarki and Kasuwa, which indicates faecal contamination or unhygiene quality of these samples (Frazier and Westhoff, 1978) that may lead to diarrheal infections of their regular consumers (Enabulele and Uraih, 2009). The presence of *E. coli* in these samples might arise from the use of contaminated water and materials during the preparation of their respective slurry and samples respectively.

Bacillus spp. was isolated in dried beef and *Kilishi* samples from Laboratory, Sabon Gari, Tudun Wada and Zaria City, indicating that these samples can cause food poisoining, an index of bacteriological quality (WFP, 2012; Okonko *et al.*, 2013). The presence of *Bacillus* spp. in these samples may be as a result of meat contamination with dust before being processed into *Kilishi*, seeing that their spores are known to endure heat treatment as reported by Busta and Foegeding (1986). *Klebsiella* spp. was then isolated in *Kilishi* samples from Tudun Wada, Zaria City, Malali, Anguwan Sarki and Kasuwa respectively. These samples may be detrimental to human with respect to *Klebsiella* spp. bacteria. Therefore, regular consumption of these samples by their consumers may lead to pneumonia and infections of the urinary tract, bloodstream and other parts of the body (Busta and Foegeding, 1986). The presence of *Klebsiela* spp. in these samples may be because of dust contamination.

These results of the bacteria isolates are similar to the studies carried out by some researchers: Igene and Abudu (1984) who identified *S. aureus, E. coli, Bacillus* spp. and *Klebsiella* spp. in *Tsire* beef products. Ogunsola and Omojola (2008), who identified *S. aureus, Bacillus* spp. and *Klebsiella* spp. in *Kilishi*. Adzitey *et al.* (2011) who identified *Salmonella*, *E. coli* and *Staphylococcus* in beef. Contrary to this study, Okonko *et al.* (2013) did not report the present of *S. aureus* and *E. coli* in *Kilishi*, while Odey *et al.* (2013) reported *Streptococcus* spp., *Pseudomonas* spp. and *Proteus* spp. in beef and *Kilishi* respectively.

Fungi genera of *Aspergillus* spp. were identified in dried beef and *Kilishi* from Sabon Gari, Tudun Wada, Anguwan Sarki and Kasuwa. *Penicillium* spp. were identified in dried beef and *Kilishi* samples from Laboratory, Sabon Gari, Anguwan Sarki and Kasuwa, while *Geotrichum candidum* were identified in *Kilishi* samples from Malali, Anguwan Sarki and Kasuwa respectively. This study of fungi isolates agreed with the findings of some authors: Pinho and Furlong (2000) who reported *Penicilium* in *Pizza* beef products. Oladejo and Adebayo-Tayo (2011) who reported *Geotrichum* spp. in *Banda* beef products. Laciakova *et al.* (2004), and Faleye and Fagbohun (2012) who reported the presence of *Asperillus* spp. and *Penicilium* spp. in *Tinco* beef and meat products respectively. Compared to this study, Faleye and Fagbohun (2012) did not report the presence of *Geotrichum* spp. but reported *Absidia candidus* in *Tinco* beef, while Laciakova *et al.* (2004) reported the presence of *Fusarium* spp., *Rhizopus nigricans* and *Fusarium monoliforme* in meat products. The presence of these fungi species in some of these study samples may be as a result of air borne dust circulation in the atmosphere during processing (Amusa *et al.*, 2002).

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 Conclusion

Commercial producers prepared six samples of sun-dried *Kilishi*, from the same beef source in the month of August. These samples were prepared and collected for analysis from their production spots in Kaduna State of Nigeria viz: Kasuwan Mata-Sabon Gari in Zaria, Yan Awaki-Tudun Wada in Zaria, Durumi Maigarke-Zaria City in Zaria, Raba Road-Malali in Kaduna, Sultan Bello Road-Anguwan Sarki in Kaduna and Kasuwa-Jos Road in Kaduna. From the same beef source, an oven-dried *Kilishi* and oven-dried beef sample were prepared from the laboratory and collected for the analysis. Standard methods by AOAC were used for the proximate and microbiological analysis with app.ropriate equipments. Flame photometer (PF9) and VARIAN AAS (AA500) were used in assessing the concentrations of the mineral contents and heavy metals in the samples respectively. The level of mineral elements, heavy metals and microbiological parameters in all the samples were determined and assessed by comparing the results with the permissible limits.

The study revealed that processing meat into *Kilishi* in Kaduna State of Nigeria improves the percent proximate parameters of the products thus making it nutrient dense. The results show that the profile of proximate parameters in the study samples is protein > crude fat > ash > moisture > carbohydrate > crude fibre. The high protein content results demonstrate the value and potential of *Kilishi* as a high protein food product. *Kilishi* from Kasuwa had the highest protein content and had the lowest values of fat, ash, moisture and carbohydrate. *Kilishi* prepared from the Laboratory that served as control seems to give the best results in trms of its effect on moisture

and fat by lowering the moisture and fat contents, and had lower value of protein contents.

The present results showed that the mineral elements (Na, Ca, Mg, P and K) found were excessively in all the samples in the profile of K > P > Na > Mg > Ca. *Kilishi* samples contained the highest values of Na, P and K contents while the highest values of Ca and Mg contents recorded were from dried beef. The Laboratory (control) *Kilishi* had the lowest Ca, Mg and P contents. From the study samples, the highest Na and Mg contents were determined in *Kilishi* from Kasuwa, highest Ca content was determined in *Kilishi* from Tudun Wada and *Kilishi* from Malali had the highest P and K contents, while the lowest mineral contents were determined in Sabon Gari *Kilishi*. The study reveals that all the samples are good sources of macro elements that are safe for consumption except in cases where their deficiencies produce diseases.

The order of the levels of the heavy metals obtained from the six study samples is Fe > Zn > Ni > Pb > Mn > Cu > Co while Cd and Cr were below detection limits. Levels of each trace essential elements of heavy metals (Fe, Cu, Zn, Mn and Co) in both the study and in the Laboratory *Kilishi* samples differed significantly among the different samples except for *Kilishi* from Sabon Gari and Zaria City that Co concentrations were below detection limits. The concentrations of Fe and Zn in this study were above their threshold effects levels, which show that consumption of these samples have accumulated unsafe levels of Fe and Zn. Copper concentrations in this study shows that consumption of these ssamples is safe since they were below its threshold effects levels. The levels of Co contents were below its threshold effects levels in the Laboratory (control) *Kilishi*, while Co contents were below detection limits in *Kilishi* samples from Sabon Gari and Zaria City. Values of Mn concentrations show that only Laboratory and Malali *Kilishi* samples had Mn below

its threshold effects levels, which indicates these samples are safe in respect to Mn hazards. From these results, Tudun Wada *Kilishi* had the highest value of Fe while Anguwan Sarki *Kilishi* had the lowest. It also shows that Sabon Gari *Kilishi* had the highest Cu and Zn values, Zaria City *Kilishi* had the highest Mn value and Anguwan Sarki *Kilishi* had the highest Co value, while the Laboratory (control) *Kilishi* had the lowest concentrations of Cu, Zn, Mn and Co, and it can be concluded that the study areas receive more of these metals. From the results of this study, the concentrations of Pb and Ni in this study exceeded their threshold effects levels, and this indicates that it is potentially harmful to consume these samples. The highest concentration of Pb found was in Zaria City *Kilishi* while the lowest Pb value found was in Malali *Kilishi*. *Kilishi* from Sabon Gari had the highest Ni content value while the lowest Ni value obtained was from Laboratory *Kilishi*.

The microbiological results revealed that the values of *Staphylococcus* counts in this study are above the threshold effects levels, and it indicates that it is unsafe to consume these samples. Again, comparison of values for Coliform counts with the FAO/WHO limits show that it is unsafe to consume *Kilishi* samples from Tudun Wada, Zaria City, Malali, Anguwan Sarki and Kasuwa due to their high Coliform counts levels. However, the results of Mesophillic aerobic counts and fungi counts were below the threshold effects levels, which show that consumption of these samples is safe. The presence of bacteria species of *Staphylococcus aureus*, *Bacillus* spp., *Salmonella* spp., *Klebsiella* spp. and *Escherichia coli* and fungi species of *Aspergillus* spp., *Penicillium* spp. and *Geotricum candidum* were confirmed in some samples with *Staphylococcus aureus* predominantly detected in all the samples. Nevertheless, the Laboratory, control *Kilishi* sample was found to be free from

Salmonella spp., *Escherichia coli, Aspergillus* spp. and *Geotrichum candidum*, and thus had less microbial contaminants.

6.2 Recommendations

Further work should be carried out at the study area to investigate for mercury and arsenic heavy metals that might be present, known to be highly toxic metals to ascertain their concentrations.

Future studies to evaluate chemical contaminants such as pesticides and herbicides, and organic contaminants such as nitrates, phosphates, sulfates and chlorides, that are ubiquitous in the environment should be carried out to ascertain the levels of their contaminations in *Kilishi* products.

Again, studies to analyzed the spices and condiments used for the production of *Kilishi* meats should be carried out to ascertain their levels, whether they are the source of the *Kilishi* contaminants.

Kilishi produced in other areas in Nigeria where there is high traffic density should be evaluated for heavy metals concentrations and microbiological contamination because environmental pollution might be high in these areas.

Government should take measures to ensure good hygienic and sanitary standard at the various slaughtered houses, abbatoir and *Kilishi* processing spots as well as good hygienic practices by the producers that are probable contributors to the heavy metals and microbial contamination on the products.

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Appendix I: Ingredients Proportion (g/100g) used for Spice Slurry in *Kilishi* Production (Ogunsola and Omojola, 2008)

English name	Scientific name	Local name (Hausa)	Proportion
Black pepper	*Piper guineese*	(Masoro)	3.40
Red pepper	*Capsicum frutescens*	(Barkono)	1.90
Sweet pepper	*Capsicum annum*	(Tattasai)	1.90
Alligator pepper	*Afromomum meliginata*	(Chitta miya)	2.10
Cloves	*Eugenia caryophillis*	(Kanuferi)	2.60
Ginger	*Zingiber officinale*	(Chitta)	3.80
African nutmeg	*Monodora myristica*	(Gujiya dan miya)	0.40
Onions	*Allium cepa*	(Albasa)	8.40
Garlic	*Allium sativum*	(Tafarnuwa)	0.10
Maggi cube	*Bullion*	(Magi)	5.80
Table salt	Sodium chloride	(Gishiri)	1.70
Curry	*Fagara xanthoxyloides*	(Kori)	0.70
Sugar	Sucrose	(Sukari)	2.50
Groundnut paste	*Aradus hypogeal*	(Tunkusa)	28.50
Water		(Ruwa)	36.20

Appendix II: Instrumental parameters for AAS Elemental Analysis

Metal	Wavelength (nm)	Lamp Current (mA)	Gas mixture (2300^{o}C)	Flow rate (L/min)	Slit width (nm)	Burner Height (mm)
Na	336.5	5	Acetylene	2.3	0.2	4
K	283.3	5	Acetylene	1.5	0.7	5
P	279.5	8	Acetylene	2.3	0.2	4
Ca	422.7	3	Acetylene	1.2	0.5	4
Mg	285.2	4	Acetylene	1.1	0.5	4
Fe	248.3	30	Acetylene	2.0	0.2	4
Cu	324.8	15	Acetylene	2.0	0.7	4
Zn	213.9	15	Acetylene	2.0	0.7	4
Co	240.8	30	Acetylene	2.0	0.2	4
Mn	279.5	20	Acetylene	2.0	0.2	4
Pb	283.3	30	Acetylene	2.0	0.7	5
Cd	228.8	4	Acetylene	2.0	0.7	4
Cr	357.9	25	Acetylene	2.5	0.7	4
Ni	232.0	25	Acetylene	2.0	0.2	4

Appendix III: Means and Standard Deviations in Mineral Contents ($mgkg^{-1}$) of Dried

Beef and its *Kilishi* Samples

Sample code	Na	Ca	Mg	P	K
RB	785.00 ± 7.07^{h}	564.30 ± 0.51^{a}	474.55 ± 0.01^{a}	3,015.00 ± 7.07^{g}	5,405.00 ± 7.07^{g}
CL	5,515.00 ± 7.07^{d}	337.51 ± 0.25^{h}	357.51 ± 0.01^{h}	4,805.00 ± 7.07^{f}	9,005.00 ± 7.07^{b}
SG	2,451.00 ± 1.41^{g}	368.26 ± 0.25^{g}	363.19 ± 0.15^{g}	5,505.00 ± 7.07^{e}	6,015.00 ± 21.21^{f}
TW	3,655.00 ± 7.07^{f}	459.55 ± 0.04^{b}	397.01 ± 0.01^{e}	6,810.00 ± 14.14^{c}	7,805.00 ± 7.07^{c}
ZC	6,220.00 ± 14.14^{c}	380.20 ± 0.01^{e}	406.83 ± 0.04^{d}	7,545.00 ± 7.07^{b}	6,755.00 ± 7.07^{e}
MK	4,440.50 ± 0.71^{e}	371.54 ± 0.06^{f}	450.64 ± 0.04^{c}	8,915.00 ± 7.07^{a}	9,610.00 ± 14.14^{a}
UK	7,430.50 ± 0.71^{b}	421.60 ± 0.01^{d}	379.46 ± 0.05^{f}	6,115.00 ± 7.07^{d}	9,015.00 ± 21.21^{b}
KK	8,281.50 ± 2.12^{a}	422.22 ± 0.29^{c}	452.42 ± 0.01^{b}	8,910.00 ± 14.14^{a}	7,010.00 ± 14.14^{d}
MEAN	4,847.31	415.64	410.21	6,452.50	7,202.50
CV	0.15	0.62	0.02	0.14	0.09
SE	7.14	0.26	0.06	9.26	6.27
RDI	2,400.00	1,300.00	420.00	1,250.00	4,700.00

RB = dried raw beef, CL = Laboratory *Kilishi*, SG = Sabon Gari *Kilishi*, TW = Tudun Wada *Kilishi*, ZC = Zaria City *Kilishi*, MK = Malali Kaduna *Kilishi*, UK = Anguwan Sarki Kaduna *Kilishi*, KK = Kasuwa Kaduna *Kilishi*, M = total mean, CV = coefficient variance, SE = standard error of means, RDI = Recommended daily intake (mg/day).

a-f: means along the same column with different superscript are significantly different ($P < 0.05$)