MICROBIAL BURDEN AND ANTIMICROBIAL SUSCEPTIBILITY OF ISOLATES FROM BEAN CAKES SOLD IN MINNA METROPOLIS, NIGERIA

BY

SHUAIB Suaburat
MTECH/SLS/2017/7080

DEPARTMENT OF MICROBIOLOGY

FEDERAL UNIVERSITY OF TECHNOLOGY

MINNA

August, 2021

ABSTRACT

Bean cakes are ready-to-eat deep-fried cowpea paste. It is commonly known as àkàrà (in Yoruba language) or kosai (in Hausa language) in Nigeria. The objectives of this study was to isolate and characterize the microorganisms associated with bean cake sold in Minna metropolis. Analysis of fried bean cakes was carried out to isolate and characterize the microorganisms associated with bean cakes sold in Minna Metropolis. The bacteria and fungi isolated were characterized using biochemical and molecular methods. Antibiotic susceptibility testing was carried out on the isolates to identify the antibiotic resistance profile, associated hazards and control measures were identified. The isolates identified were *Aspergilus niger, Bacillus subtilis, Micrococcus roseus, Staphylococcus aureus, Klebsiella pneumoniae* and *Escherichia coli.* The total viable bacterial counts ranged from 0.00±0.00cfu/g in Dutsen kura to 2.58±0.10cfu/g in F-layout while the coliform counts ranged from 0.00±0.00 cfu/g in Bosso and Dutsen kura to 1.75±0.11cfu/g in Kpakungu and fungal counts ranged from 0.00±0.00 in Tunga and Bosso to 2.66±0.18cfu/g in Maitumbi. *Staphylococcus aureus, K, pneumoniae* and *M. roseus* were susceptible to 45.45% of the antibiotics used while *E. coli, B. subtilis* and *A. niger* were susceptible to 31.98%, 27.27 % and 60% of the agents used. Critical control points were identified as milling and water used in mixing the paste. The results suggested that some degree of control can be exercised over the microbial hazard of kosai to prevent, eliminate or reduce microbial load to acceptable levels. There should be enlightenment campaign created amongst sellers and consumers of bean cakes.

TABLE OF CONTENTS

Content		Page
Cover Page		i
Title Page		ii
Declaration		iii
Certification		iv
Dedication		v
Acknowledgements		vi
Abstract		viii
Table of Contents		ix
List of Tables		xvii
List of Figures		xix
List of Plates		xx
List of Abbreviation		xxi
CHAPTER ONE		1
1.0	INTRODUCTION	1
1.1	Background to the Study	1
1.2	Statement of the Research Problem	3
1.3	Justification for the Study	4
1.4	Aim and Objectives of the Study	4
CHAPTER TWO		**6**
2.0	LITERATURE REVIEW	6

2.1 Description of Cowpea 6

2.1.1 Production of cowpea 7

2.1.2 Consumption of cowpea 8

2.1.3 Nutritional quality of cowpea 9

2.1.4 Anti-nutritional properties of beans 11

2.2 Bean Cake 12

2.2.1 Types of bean cake 12

2.3 Common Microbial Contaminants of Bean Cake 12

CHAPTER THREE **15**

3.0 MATERIALS AND METHODS 15

3.1 Study Area 15

3.2 Sample Collection 16

3.3 The Sale Points 16

3.4 Determination of Microbial Contamination Level 19

3.5 Characterization and Identification of Isolates 19

3.6 Identification and Characterization of Fungal isolates 22

3.7 Aseptic Preparation of Bean cake in the Laboratory 23

3.8 Molecular Identification of Isolates 25

3.8.1 DNA extraction 25

3.8.2 Polymerase chain reaction 25

3.8.3 Integrity of amplified DNA 26

3.8.4 Purification of amplified product 26

3.8.5 Sequencing of the isolates 27

3.9 Antimicrobial Susceptibility Testing 27

3.10 Antifungal Susceptibility Testing 27

3.11 Identification of Hazards and Critical Control Points in the Preparation of Fried Bean Cakes 28

3.12 Determination of Frequency of Occurrence of Microbial Isolates from Bean Cakes 28

3.13 Data Analysis 28

CHAPTER FOUR **28**

4.0 RESULTS AND DISCUSSION 29

4.1 Results 29

4.1.1 Bacterial load of bean cakes sold in Minna metropolis 29

4.1.2 Coliform count of bean cakes sold in Minna metropolis 32

4.1.3 Fungal load of bean cakes sold in Minna metropolis 34

4.1.4 Cultural characteristics of bacterial isolates 36

4.1.5 Microscopic, morphological and biochemical characteristics of bacteria isolated from bean cakes 36

4.1.6 Cultural and morphological characteristics of fungal isolates from bean cakes 36

4.1.7 Frequency of occurrence of the microorganisms isolated from bean cakes sold in Minna metropolis 37

4.1.8 Molecular identities of the isolates 41

4.1.9 Antimicrobial susceptibility profile of the bacteria isolated from bean cakes sold in Minna metropolis 42

4.1.10 Antifungal susceptibility testing 43

4.1.11 Sources of hazards associated with bean cakes 46

4.2 Discussion 49

CHAPTER FIVE **52**

5.0 CONCLUSION AND RECOMMENDATION 52

5.1 Conclusion 52

5.2 Recommendation 52

REFERENCES **53**

LIST OF TABLES

Table		Page
2.1	Scientific classification of cowpea	6
3.3	Description of the sale points	18
4.1	Bacterial load of Bean Cakes Sold in Minna Metropolis, Nigeria	31
4.2	Coliform Counts of Bean Cakes Sold in Minna Metropolis, Nigeria	33
4.3	Fungal Load of Bean Cakes Sold in Minna Metropolis, Nigeria	35
4.4	Cultural Characteristics of the Bacterial Isolates	38
4.5	Microscopical, Morphological and Biochemical Characteristics of the Bacterial Isolates	39
4.6	Cultural and Morphological Characteristics of Fungal Isolates from Bean Cake	40
4.7	Frequency of occurrence of bacterial isolates of bean cakes sold in Minna metropolis	40
4.8	Accession number of the isolates	42
4.9	Antimicrobial susceptibility profile of the bacteria isolated sold in Minna metropolis	44
4.10	Antifungal Susceptibility Testing	45
4.11	Microbial Load of Water used for Bean Cake Production in Minna Metropolis, Nigeria	47

4.11b Microbial Load of Bean Paste used for Bean Cake Production in Minna Metropolis, Nigeria 48

LIST OF FIGURES

Figure **Page**

I Map of Niger State showing Minna Metropolis 15

II Flow chart for the preparation of Fried Bean Cake 24

LIST OF PLATES

Plate		Page
I	*Vigna unguiculata*	7
II	Picture of bean cake	16
III	Gel electrophlorograph showing the positive amplification of bacterial isolates using the 16S universal primers. A band size of about 1500bp indicated a positive amplification	41
IV	Gel electrophlorograph showing the positive amplification of sabr6 (fungi) isolate using the ITS universal primers. A band size of about 550bp indicated a positive amplification	41

LIST OF ABBREVIATION

Abbreviation	Meaning
ANOVA	Analysis of variance
AUG	Augmentin
CLD	Clindamycin
COT	Cotrimoxazole
CPX	Cephaplexin
CPX	Ciprofloxacin
CRO	Ceftriazone
CXL	Cloxacillin
DNA	Deoxyribo-nucleic acid
ERY	Erythromycin
GEN	Gentamycin
HACCP	Hazard Analysis and Critical Control Point
IITA	International Institute of Tropical Agriculture
mRNA	messenger RNA
OFL	Ofloxacin
PFX	Pefloxacin
SEM	Standard Error of Mean

VPM Vogues-Proskauer Medium

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Cowpea (*Vigna unguiculata*) is a legume popularly known as beans in West Africa (Moutaleb *et al.,* 2017). Cowpea is nutritious and contains protein, vitamins and minerals. Cowpea has about 25% protein, making it extremely valuable for people who cannot afford proteins from animal sources such as meat and fish (Appiah *et al.,* 2011; Oumarou *et al.,* 2017). Cowpea can be processed as flour, paste, deep fried cake (akara) or steam bean pudding (moi moi) and bean soup eaten in several Western and Central African countries (Eke-Ejiofor and Kporna, 2019).

Bean cake known as "àkàrà" in Yoruba, "kosai" in Hausa, is a popular food in Nigeria, Ghana, Togo, Benin, Mali and Gambia (Aviara *et al.,* 2018). It forms part of the diet of most ethnic groups in Nigeria. Nigerians usually eat it as breakfast with 'ogi', or lunch with 'gari' or even dinner with 'eko'. Akara is a traditional African food made by deep frying cowpea paste that has been whipped and seasoned with salt, pepper, onions and other optional ingredients. The outer crust of Akara is crispy and the interior is spongy like bread. It is considered to be the most commonly consumed cowpea-based food in West Africa. Akara is made mainly from cowpea and other sources like maize, rice and flour. It can be fried with vegetable oil, palm oil, and other edible oils (Aviara *et al.,* 2018).

Foodborne pathogens are microorganisms found in foods that are capable of causing diseases when consumed. Some foodborne microbes make people ill by forming toxins in foods that affect the gut or the neurological system. Foodborne pathogens include *Salmonella typhimurium, Escherichia coli, Listeria monocytogenes, Clostridium perfringens, Bacillus subtilis, Bacillus cereus* and *Klebsiella pneumoniae*. Foodborne pathogens associated with

bean cake according to the findings of Lateef *et al.* (2016) are *Escherichia coli, Staphylococcus aureus, Staphylococcus epidermidis, Citrobacter freundii, Serratia marcescens, Proteus vulgaris, Bacillus cereus, Streptococcus pyogenes, Aspergillus niger, Shigella* spp and *Bacillus* spp.

The contamination of bean cake is largely due to post-processing operations such as unhygienic handling of bean cake with bare hands, exposing bean cake without covering it, talking when selling bean cake, using old newspapers that were not properly kept among others. These post-processing operations can be abated through the use of quality water, high level of personal hygiene and hygienic production materials (Toledo, 2018).

Examples of foodborne diseases caused by these organisms include; Salmonellosis and foodborne illness caused by *E. coli* O157:H7. Other foodborne diseases include: Amoebiasis, cholera, diarrheagenic *E. coli*, giardiasis, listeriosis, marine toxins shigellosis, travelers' diarrhea, trichinosis (*trichinellosis*), typhoid, *Vibrio parahaemolyticus* and *Vibrio vulnificus* infection. Symptoms of these diseases include nausea, vomiting, abdominal cramps and diarrhea.

One of the major problems associated with bean cake is its susceptibility to various types of spoilage such as staling, rancidity and ropiness, soon after its production. Bean cake starts to stale the minutes it leaves the fryer which makes its outer surface to become firm, harsh, opaque and crumblier. Bean cake has a poor shelf-life which has been attributed to its fat and high moisture content (Oumarou *et al.,* 2017). Associated with the fat content is lipid oxidation while high moisture content in the product predisposes carbohydrate and protein in it to fermentation and putrefaction respectively causing ropiness by *Bacillus subtilis* (Oumarou *et al.,* 2017).

In view of the problems associated with the consumption of bean cake, particularly if basic hygienic considerations are not observed after its production, this study was designed to evaluate the microbiological quality of bean cake from some selected sellers within Minna metropolis. The bacterial isolates were subjected to antimicrobial sensitivity test and molecular characterization with the view of establishing the public health implications of their occurrence in bean cake. In addition, the environment, raw materials and the procedure for preparing bean cake were monitored to identify sources of hazards and possible critical control points by applying the hazard analysis and critical control point (HACCP) strategy.

1.2 Statement of the Problem

The presence of microorganisms in food has always been attributed to contamination through water, soil, processing equipment, contact surfaces and the food handlers (Toledo, 2018). Improper handling of food is responsible for most cases of food borne diseases and cross contamination (Toledo, 2018). About 1 in 10 people in the world fall sick after eating food contaminated through improper farming, processing and preservation. In Nigeria, more than 200,000 persons die of food poisoning annually caused by the consumption of contaminated foods (Aviara *et al.,* 2018).

Bean cake has a poor shelf life which can be attributed to its fat and high moisture content. Associated with the fat content is lipid oxidation while high moisture content in the product predisposes carbohydrate and protein in it to fermentation and putrefaction respectively, causing ropiness by *Bacillus subtilis* (Alum *et al.,* 2016). The nutritional content of bean cake has predisposed it to microbial contamination. In 2010, in Bekwara Local Government Area of Cross River State, 2 children died and 122 people hospitalized as a result of food poisoning due to the consumption of moi-moi, a steamed cowpea paste delicacy (Alum *et al.,* 2016).

There are multiple sources of contamination from the environment, and contaminants could enter the food during harvest, production, storage and selling of bean cake. And as such, it is imperative that bean cake, a cowpea base delicacy be examined for the presence of contaminants.

1.3 Justification for the Study

Bean cake is a common food consumed by Western and Central African countries especially in Nigeria. Several studies have reported the contamination of bean cake by pathogenic organisms from diverse sources. Lateef *et al.* (2016) investigated the microbiological examination and identification of hazards and critical control points of akara ogbomoso. Also, Ajibola and Adelekan (2017) investigated the storage stability of deep-fried cowpea products (akara) incorporated with soy-flour and *Aframomum danielli,* while China *et al.* (2019). Proximate composition and sensory assessment of beans pudding prepared using two different cooking methods and Hence, there is a great need to determine the microbial quality and identification of hazards and critical control points of bean cake sold in Minna Metropolis.
Data generated will add to existing information on the microbiological quality of bean cake.

1.4 Aim and Objectives of the Study

The aim of this study was to isolate and characterize the microorganisms associated with bean cake sold in Minna metropolis.

The specific objectives of the study were to:

i. isolate and characterize the microorganisms associated with bean cake sold in Minna metropolis.

ii. compare the microbial quality of the bean cakes obtained from the market and the aseptically prepared bean cake in the laboratory.

iii. investigate the susceptibility of these microorganisms to antimicrobial agents.

iv. identify the hazards and critical control points in the production of bean cakes.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Description of Cowpea

Cowpea (*Vigna unguiculata*) is a legume popularly known as beans, black-eye pea, southern pea, yardlong bean, catjang, crowder pea and waake in the Northern part of Nigeria (Moutaleb *et al.*, 2017). They are composed of a cotyledon, germ and seed coat with a testa and hilum. The seed coat is where the coloured pigment is contained and it can be removed to reveal a white seed (cotyledon). Beans is an annual herbaceous plant cultivated as a food source to human and livestock as well as cash crop to both subsistence and large-scale farmers of most African countries (Witthof and Hefni, 2016). Cowpea is nutritious and contains protein, vitamins and minerals. Cowpea has about 25% protein, making it extremely valuable to people who cannot afford proteins from animal sources such as meat and fish (Oumarou *et al.,* 2017).

Presently, Nigeria is the largest producer of cowpea in the world; it is a crop that complements carbohydrate such as sorghum, corn and millet, thereby improving dietary provisions of households (Olu *et al.*, 2017). Due to its tolerance for sandy soil and low rainfall, it is an important crop in the semi-arid regions across Africa and other continents.

Table 2.1: Scientific classification of cowpea

Taxonomic Group	Plant
Kingdom	Plantae
Order	Fabales
Family	Fabaceae
Genus	*Vigna*
Species	*Unguiculata*

Source: (Olu *et al.,* 2017).

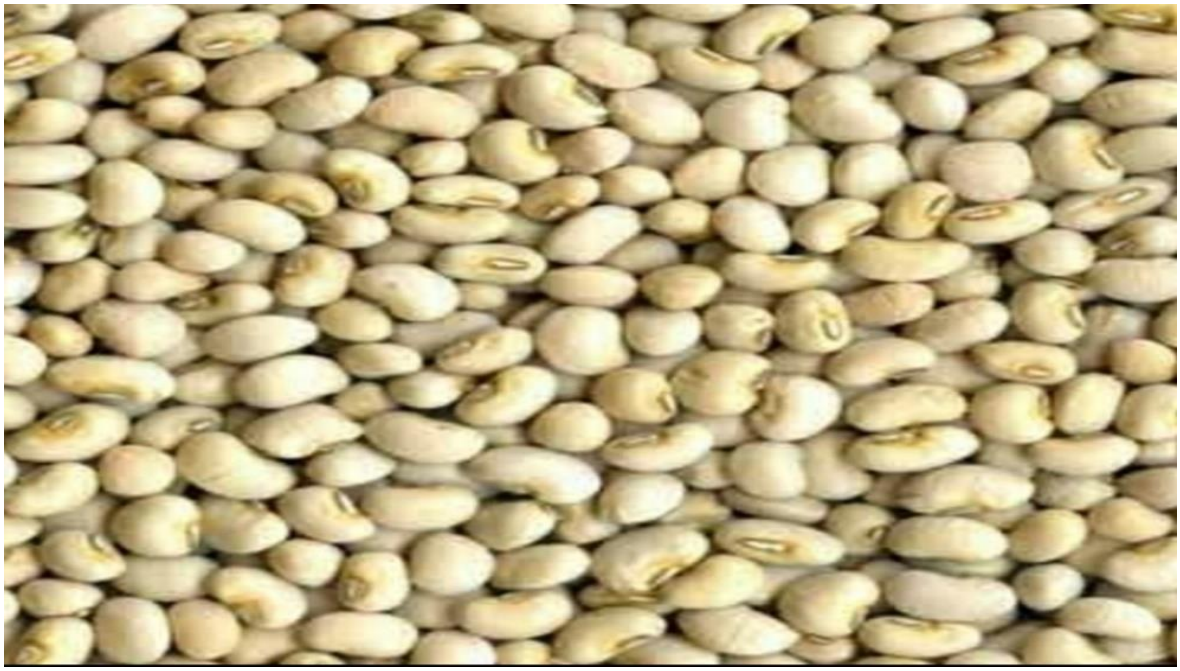

Plate I: *Vigna unguiculata* (Source: This study)

2.1.1 Production of Cowpea

The history of cowpea dates to ancient West African cereal farming, 5-6 thousand years ago, where it was closely associated with the cultivation of sorghum and millet. Cowpea is a key staple food for the poorest sector of many developing countries of the Torrid Zone. In 1981 production was estimated at 2.27 million tons from 7.7 million hectares, with Africa producing two-third. In 2001 total worldwide production was estimated at 3.3 million tons of the dry grain with Africa producing 64% (Olu *et al.,* 2017). West Africa is the region with the largest production and consumption of cowpea. Conservative estimates suggest that greater than 12.5 million hectres (ha) are planted annually to cowpea around the world and of this area, about 9.8 million ha are planted in West Africa. The primary cowpea producing countries in West Africa are Nigeria, Niger, Mali, Senegal, Burkina Faso and Ghana (Alum *et al.,* 2016).

Cowpea has been cultivated in the southern parts of the United States since the early eighteenth century. It is estimated that 80,000 ha of cowpeas are grown in the United States each year.

The United States is the only developed country producing large amounts of cowpea where they are grown primarily for dry seed in California and West Texas (Eke-Ejiofor and Kporna, 2019). Cowpea cultivars that are popular in Nigeria can be classified into three major groups, namely, black-eyed beans, honey beans and cream types. The black-eyed beans and the cream types are the most popular and widely used in Nigeria. Cowpeas thrive in poor dry conditions, growing well in soils up to 85% sand (Olu *et al.,* 2017). This makes them a particularly important crop in arid, semi-desert regions where not many other crops will grow. Its nitrogen-fixing ability means that as well as functioning as a sole crop, the cowpea can be effectively intercropped with sorghum, millet, maize, cassava, or cotton (Olu *et al.,* 2017). The optimum temperature for cowpea growth is 30 °C (86 °F), making it only available as a summer crop for most of the world. It grows best in regions with an annual rainfall between 400 and 700 mm (16 and 28 in). The ideal soils are sandy and it has better tolerance for infertile and acid soil than most other crops.

2.1.2 Consumption of Cowpea

The most important part of the crop is the dry grain which is commonly boiled and eaten as beans porridge. Cowpea can be processed as flour, paste, deep fried cake (akara) or steam bean pudding (moi moi) and bean soup eaten in several Western and Central African countries (Eke-Ejiofor and Kporna, 2019). In the United States legume consumption is extremely low (5 g/day) but legumes provide half or more of the dietary protein in developing countries, about 10-150 g/day of legumes is consumed in India and Sub-Saharan Africa and 50-89 g/day in Latin America (Olu *et al.,* 2017).

The difference in consumption between developing and developed countries is because of a lack of modern food technologies for converting legumes to attractive, convenient foods. Beans are generally boiled over fire and eaten. The western food processors need to develop technologies to convert legume seeds to tasty, nutritious foods which will be accepted by the

developed countries. In the United States, they are also utilized for fodder and a quick-growing cover crop under a wide range of conditions. They are able to fix nitrogen and provide a high proportion of their own nitrogen requirements, and they also leave a fixed nitrogen deposit in the soil of up to 60-70 Kg/ha for the succeeding crop (China *et al.,* 2019).

2.1.3 Nutritional quality of Cowpea

Akajiaku *et al.* (2014) and Sakpo and Osundahunsi (2016) reported that cowpea contains carbohydrate in the form of starch and dietary fiber which helps to prevent constipation, bowel problems and piles. International Institute of Tropical Agriculture (IITA, 2012) has shown that cowpea is a good source of plant-based protein that has been identified as a meat alternative with a percentage of 21 to 25% protein by weight higher than other sources of vegetable protein which has a low glycemic index. This makes them an ideal food for the management of insulin resistance diabetics (China *et al.,* 2019).

Cowpea also contains potassium, a mineral that promotes healthy blood pressure levels, copper, phosphorous, magnesium, iron which makes them important for vegetarians and vegans who do not get an animal source of iron. It has water–soluble vitamins like thiamin and folic acid and good sources of riboflavin and vitamin B6 (Eke- Ejiofor and Kporna, 2019). China *et al.* (2019) reported that in countries such as Nigeria, and most of the Sub-Sahara countries, animal products representing high concentration and quality of protein are either too expensive or simply unaffordable, thus increasing the dependence on cereal grains, roots and tuber crops. However, the growth in the dietary share of cowpea has been constrained by high preparation time, labour requirements, cooking methods and undesirable product characteristics. It also includes beany flavour (China *et al.,* 2019).

Cowpea's superior nutritional attributes, versatility, adaptability and productivity led to it being chosen by the U.S. National Aeronautical and Space Administration as one of few crops worthy

of study for cultivation in space stations. The major storage proteins are globulins (70%) and glutelins. These proteins are considered the ‘power houses’ of legumes because they provide most of the essential amino acids to the diet (Preetha and Narayayan, 2020).

Total carbohydrate varies from 56-68% with starch contributing 32-48%. The amount of amylase present influences starch solubility, lipid binding and many functional properties, such as swelling and solubility, water absorption, gelatinization and pasting, that affect cooking practices and acceptability Sucrose is the most abundant sugar, followed by stachyose, verbascose and raffinose. Cowpeas contain large amounts of crude fiber, of which cellulose is a major component. They are an excellent source of thiamin and niacin and also contain reasonable amounts of other water-soluble vitamins, riboflavin, pyridoxine and folacin. In addition they supply the essential minerals, calcium, magnesium, potassium, iron, zinc and phosphorus (Osundahunsi, 2016).

Cowpea seeds provide a rich source of proteins and calories, as well as minerals and vitamins This complements the mainly cereal diet in countries that grow cowpeas as a major food crop a seed can consist 25% protein and has very low fat content Cowpea starch is digested more slowly than the starch from cereals, which is more beneficial to human health. (Gonçalves *et al.,* 2016). The grain is a rich source of folic acid, an important vitamin that helps prevent neural tube defects in unborn babies (Witthöft and Hefni, 2016).

The cowpea has often been referred to as "poor man's meat" due to the high levels of protein found in the seeds and leaves (Hamid *et al.,* 2016). However, it does contain some anti-nutritional elements, notable phytic acid and protease inhibitors, which reduce the nutritional value of the crop (Gonçalves *et al.,* 2016). Methods such as fermentation, soaking, germination and debranning are used to combat the anti-nutritional properties of the cowpea by increasing the bioavailability of nutrients within the crop (Owade *et al.,* 2019). Although little research

has been conducted on the nutritional value of the leaves and immature pods, what is available suggests that the leaves have a similar nutritional value to sweet potato leaves, while the green pods have less anti-nutritional factors than the dried seeds (Gonçalves *et al.,* 2016).

2.1.4 Anti-nutritional properties of beans

Beans contain significant amounts of anti-nutrients that inhibit some enzyme processes in the body. Phytic acid and phytates present in grains, nuts, seeds and beans, interfere with bone growth and interrupt vitamin D metabolism. Many edible beans contain oligosaccharides (particularly raffinose and stachyose), a type of sugar molecule also found in cabbage. An anti-oligosaccharide enzyme is necessary to properly digest these sugar molecules. As a normal human digestive tract does not contain any anti-oligosaccharide enzymes, consumed oligosaccharides are typically digested by bacteria in the large intestine. This digestion process produces gases, such as methane as a byproduct, which are then released as flatulence (Pottorff *et al.,* 2019).

2.2 Bean cake

Bean cake is popularly called àkàrà in yoruba and kosai in hausa. It is a popular food in Nigeria, Ghana, Togo, Benin, Mali and Gambia (Aviara *et al.,* 2018). It is very important in the diet of an average Nigerian. It is usually eaten as breakfast with ogi (pap), or lunch with gari or even dinner with eko. Akara is an African food made by deep frying cowpea paste that has been whipped and seasoned with salt, pepper, onions and other optional ingredients. The outer crust of akara is crispy and the interior is spongy like bread. It is considered to be the most commonly consumed cowpea based food in West Africa. Akara is made mainly from cowpea and other sources like maize, rice and flour. It can be fried with vegetable oil, palm oil, and other edible oils (Aviara *et al.,* 2018).

2.2.1 Types of Bean cake

There are two types of bean cake depending on the method of production, moisture content and the extent of drying. The first type is the conventional akara. It is the most popular, which is produced by a single deep frying in palm or vegetable oil and contains a lot of moisture. It cannot be stored for more than 8-10h at ambient temperature (Lateef *et al.*, 2016). The second type has low moisture content (usually ground as thick paste without water) and often deep-fried two times in hot vegetable oil, can be stored for 8-10 weeks without spoilage by microorganisms. While whipping is an important step in the preparation of conventional akara to form foam which impacts the texture of akara, the preparation of the second type does not involve whipping in any form. Also, unlike in the first type of akara, seasoning ingredients such as onion and pepper are not used in the preparation of the second type of akara (Ajibola and Adelekan, 2017).

Akara Ogbomoso represents the second group, whose name was derived from the main center of production. Akara Ogbomoso differs from the conventional akara in terms of process, product characteristics and shelf stability Its production had been an exclusive preserve of Ogbomoso sub-tribe of 'Yoruba' race, although its preparation can now be found in other towns and cities in 'Yoruba' land, Southwest Nigeria. Because of its long shelf-life, the popularity and consumption of akara Ogbomoso have increased tremendously and it can now be found in several other cities in Southwestern Nigeria (Ajibola and Adelekan, 2017).

2.3 Common Microbial Contaminants of Bean Cake

Escherichia coli (*E. coli*) naturally form part of the normal flora in the gut of humans and other animals. In fact, most *E. coli* are considered harmless to humans. However, certain pathogenic *E. coli* strains can infect the gut area and cause severe illness. Pathogenic *E. coli* infection usually causes severe diarrhea. Diarrhea is the result of the reversal of the normal net absorptive status of water and electrolyte absorption to secretion. Worldwide, there are nearly 1.7 billion

cases of diarrheal disease every year. Diarrheal disease is the second leading cause of death in children under 5 years old. Every year about 760,000 children under 5 years old die due to diarrheal diseases (Lateef *et al.*, 2016). Fortunately, diarrheal disease caused by pathogenic *E. coli* is preventable by improved environmental sanitation and is treatable by antibiotics. The treatment of diarrheal disease is generally effective with oral rehydration and maintaining electrolyte balance through the diet (Ajibola and Adelekan, 2017). Patients with severe dehydration may require intravenous rehydration and use of antidiarrheal, pain relief drugs and antibiotics can slow down the patient's symptoms. However, certain strains that can cause infections and illness become resistant to antibiotics. The pathogenic *E. coli* can be found in soil and water, usually as a result of animal fecal contamination (Preetha and Narayayan, 2020).

Staphylococcus aureus is gram positive cocci which causes staphylococcal food-borne disease (SFD). Staphylococcal food-borne disease (SFD) is one of the most common food-borne diseases worldwide resulting from the contamination of food by preformed *S. aureus* enterotoxins. It is one of the most common causes of reported food-borne diseases in the United States. Presence of pathogens in food products imposes potential hazard for consumers and causes grave economic loss and loss in human productivity via food-borne disease (Lateef *et al.*, 2016). Symptoms of SFD include nausea, vomiting, and abdominal cramps with or without diarrhea. Preventive measures include safe food handling and processing, adequate cleaning and disinfection of equipment, prevention of cross-contamination at homes and kitchen, and prevention of contamination from farm to mouth.

Aspergillus niger is a filamentous fungus growing aerobically on organic matter. In nature, it is found in soil and litter, in compost and on decaying plant material (Preetha and Narayayan, 2020). *Aspergillus niger* is less likely to cause human disease than some other *Aspergillus* species. In extremely rare instances, humans may become ill, but this is due

to a serious lung disease, aspergillosis, that can occur. Aspergillosis is, in particular, frequent among horticultural workers who inhale peat dust, which can be rich in *Aspergillus* spores. *Aspergillus niger* is one of the most common causes of otomycosis (fungal ear infections), which can cause pain, temporary hearing loss, and, only in severe cases, damage to the ear canal and tympanic membrane (Lateef *et al.*, 2016).

Klebsiella pneumoniae is an opportunistic bacterium found in various microbiological niches such as soil; the skin, intestines, and feces of mammals; and food (Preetha and Narayayan, 2020). *K. pneumoniae* has been documented to cause bacteremia, pneumonia, and urinary tract infection; the gastrointestinal carriage of *K. pneumoniae* has been said to be a predisposing factor for liver abscess and hypervirulent *K. pneumoniae* strains have emerged as a predominant cause of pyogenic liver abscess in Asia (Lateef *et al.*, 2016).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

Bean Cakes were collected from Minna Metropolis, Niger State of Nigeria. Minna is situated at 9.58^{o} North latitude, 6.55^{o}East longitude and 250.6 meters elevation above the sea level (Olusegun *et al.,* 2020).

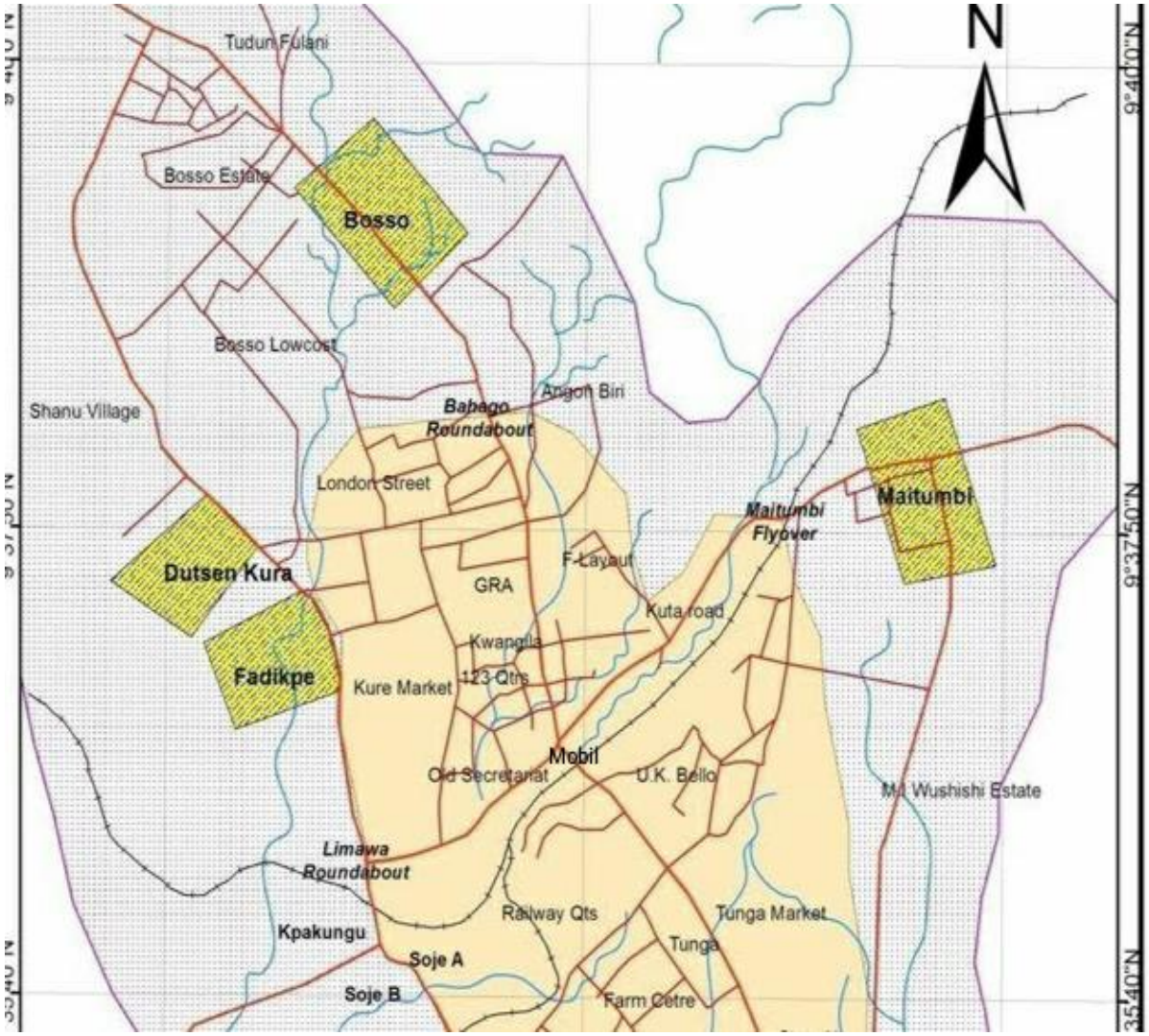

Figure II: Map of Minna Metropolis showing the selected sale points.

(Source: Olusegun *et al.,* 2020)

3.2 Sample Collection

A total of 270 bean cake balls were collected in this study using three methods; hand picking of bean cake into nylon, hand picking into clean container and picking with fork into clean container. A total of 252 balls of bean cake were collected from 14 sale points and 18 bean cake balls were prepared aseptically in the laboratory. The samples were collected and taken to the laboratory immediately for analysis. In addition, grinded bean paste and water samples were collected from these points for analyses. The samples were collected from two sales points in Tunga, Maitumbi, Mobil, F-layout, Dutsen kura, Kpagungu and Bosso areas of Minna, Nigeria. The samples were collected in the months of January, February and March, 2020. Plate II shows the picture of bean cake.

Plate II: Picture of bean cake (Source: Field Photography)

3.3 The Sale Points

The sale points visited were either located beside the road, in the motor pack or in the market. Some of the points have their location closed to a dusty road or toilet. The producers have never undergone any formal training on food production. Questionnaires were prepared and issued

to producers of bean cake. Table 3.2 shows the description of the sale points visited. The environmental hygiene of all the sale points were not good for food preparation. Most of the producers do not have any formal training on food production. Some of the producers lack personal hygiene. Their main sources of water were well, tap and water vendors. The type of packaging method used for the sale of bean cake is hand picking into nylon.

Table 3.3: Description of the sale points

Parameters/ production and selling points	TA	TB	MA	MB	FA	FB	BA	BB	KA	KB	AA	AB	DA	DB
Location	Market	Road side	Road side	Motor pack	Road side	Road side	Road side	Market	Motor pack	Road side	Road side	Market	Road side	Road side
Sources of water	Well	Well	Water vendor	Water vendor	Well	Well	Tap/Well	Well	Well	Well	Well	Well	Well	Well
Presence of Drainage/refuse dump	No	Yes	No	No	Yes	No	Yes	No	Yes	Yes	No	No	Yes	No
Level of environmental sanitation	Good	Fair	Fair	Good	Poor	Fair	Fair	Fair	Poor	Poor	Good	Good	Fair	Fair
Acquired formal training on food production?	No	No	No	No	No	No	No	No	No	No	No	No	No	No
Other remarks	Water was stored uncover	Uses bare hands to pick bean cake	Production utensils were not properly washed	Lack personal hygiene	Water left uncovered	Close to a dusty road	Produced Bean cake left uncovered	Uses bare hands to pick bean cake	Lack personal hygiene	Toilet too close to production points	Water was left uncovered	Paste was left uncovered	Lack Personal and environmental hygiene	Production Materials were not properly kept

TA: Tunga A, TB: Tunga B, MA: Mobil A, MB: Mobil B, FA: F-layout A, FB: F-layoutB, BA: Bosso A, BB: Bosso B, KA: Kpakungu A, KB: Kpakungu B, AA: Maitumbi A, AB: Maitumbi B, DA: Dutsen kura A, DB: Dutsen kura B

3.4 Determination of Microbial Contamination Level

Pour plate method of Sagar (2019) was used for the isolation, enumeration and identification of bacterial and fungal species contaminating the samples. A gram of each of the bean cake samples (mashed) were transferred into 10 mL of sterile distilled water to obtain a stock solution. A milliliter (1 mL) of the stock solution was transferred into 9 ml of sterile distilled water to obtain 10^{-1} dilution. Further dilution was made to 10^{-6}. An aliquot (1 mL) of the 4th diluent was aseptically inoculated in triplicates into freshly prepared molten nutrient agar, MacConkey agar, Salmonella Shigella agar and Manitol salt agar for bacterial count and the 3rd diluent was aseptically inoculated in triplicate into Sabouraud dextrose agar respectively. The innoculated plates were incubated for 24 hours at 37 ℃ for bacteria and at room temperature (25±2 ℃) for 48 h for fungi. The isolates were further sub-cultured repeatedly onto a fresh Nutrient agar plates for the bacteria and saboraud dextrose agar for the fungi so as to obtain pure isolates. After sub-culturing, the plates were incubated accordingly. The pure isolates were preserved in slant bottles for further characterization and identification as outlined in Bergey's Manual of Determinative Bacteriology.

The resulting colonies were counted using coulter counting chamber and calculated using the formula below:

Colony forming unit (cfu) = number of colonies x volume of diluent x reciprocal of dilution

3.5 Characterization and Identification of Isolates

The method of Bassiri, E. A (2020) was employed for the isolation and Characterization of identities of the bacterial isolates. The isolates obtained were subjected to Gram staining, Motility test, Oxidase test, Catalase test, Triple sugar iron agar test, Indole test, Urease test, Hydrogen sulphide production test, Citrate utilization test, Methyl red test and Voges-prokauer test.

3.5.1 Gram staining

Grease-free glass slides were used to prepare smear and the slides were placed on the staining rack. The smear was covered with crystal violet stain and left for 1 minute then washed carefully under running tap water. The smear was flooded with Gram's iodine solution and left for 1 minute. The iodine was drained off the slides and washed in a gentle stream of tap water. The slides were flooded with alcohol for 30 seconds and washed under running tap water then drained completely. The slides were counterstained with safranin for 1 minute and washed in a gentle stream of tap water until no color appears in the effluent then the slides were blotted dry with absorbent paper and observed under the microscope. Gram-positive bacteria appeared dark purple while Gram-negative bacteria appeared pale to dark red.

3.5.2 Motility test

From fresh overnight liquid cultures, a straight wire loop was used to innoculate tubes containing the medium by stabbing straight halfway the tubes. The inoculated tubes were incubated at 37 ^{0}C for 24 h. The tubes were observed for the presence or absence of growth along the line of stab. Motile bacteria grew along the line of stab and diffused into the medium with turbidity while non-motile bacteria grew only along the line of stab and did not diffuse into the medium with no turbidity.

3.5.3 Oxidase test

Colonies from 24-hour cultures were placed on filter papers and a drop of oxidase reagent was added onto each filter paper and examined within 10 seconds. Oxidase-positive bacteria developed bluish-purple colour while oxidase-negative bacteria did not develop blue colour.

3.5.4 Catalase test

A sterile wire loop was used to pick colonies from 24-hour cultures on to dry glass slides. A drop of 3 % hydrogen peroxide (H_2O_2) was placed on each glass slide and observed for the

evolution of air bubbles. Catalase-positive bacteria produced copious active bubbles while catalase negative bacteria produced few bubbles or none.

3.5.5 Urease test

The surface of Urea agar slants was streaked with 24-hour broth cultures. The cap of the tubes was left on loosely and incubated at 37 ^{0}C for 48 hours then examined for colour change. Urease positive-bacteria developed a magenta to bright pink colour in 24-hours while Urease negative-bacteria did not develop colour change.

3.5.6 Citrate utilization test

Inocula picked from the centre of a well isolated colony of 24 h cultures were streaked on slant tubes containing simmon citrate agar. The slant tubes were incubated at 37^{0}C for up to 4 days and observed for colour change along the slants. Bacteria that utilized citrate showed growth with colour change from green to intense blue along the slants while bacteria that did not utilize citrate showed no growth and no colour change (slants remained green).

3.5.7 Hydrogen sulphide production test

Inocula picked from colonies of 24 h bacterial cultures were seeded in tubes containing Kligler iron agar (KIA) by straight stabbing to a depth of 2 cm. The tubes were incubated at 37 ^{0}C for 48 h and the tubes were observed for colour change for H_2S production. Bacteria that produced H_2S turned the medium black while bacteria that did not produce hydrogen sulfide did not turn the medium black.

3.5.8 Methyl red test

Prior to inoculation, the media was allowed to equilibrate to room temperature (25±2 ℃). Inocula from 24 h bacterial cultures were transferred into tubes containing Methyl red medium. The tubes were incubated at 37 ^{0}C for 24 h. Following 24-hour of incubation, 1mL of the broth

was transferred into each clean test tube. The remaining broth were reincubated for an additional 24 h. Two drops of Methyl red indicator was added to each aliquot and observed for colour change immediately. Red colour indicated positive reaction while yellow colour indicated negative reaction.

3.5.9 Voges-Proskauer test

Prior to inoculation, the Vogues-Proskauer (VP) medium was allowed to equilibrate to room temperature (25±2 ℃). Inocula from 24 h bacterial cultures were inoculated into the tubes containing VP medium. The tubes were incubated at 37 ^{0}C for 24 h. Following 24 h of incubation, 1 mL of the broth was dispensed into each clean test tube. The remaining broth were re-incubated for an additional 24 h. Six drops of 5% alpha-naphthol were added to each aliquot and homogenized. Two drops of 40% potassium hydroxide were added to each aliquot and agitated. The tubes were agitated vigorously for 30 minutes and observed for colour change. A pink-red colour at the surface of the tubes indicated a positive reaction while absence of pink-red colour at the surface of the tubes indicated a negative reaction.

3.5.10 Indole test

Test tube containing 4 mL of tryptophan broth each were sterilized. The tubes were inoculated aseptically with inoculum from 24 h bacterial cultures. The tubes were incubated at 37 ^{0}C for 48 h. Kovac's reagent 0.5mL was added to each broth culture and observed for the presence or absence of ring. Formation of a pink to red colour (cherry red ring) in the reagent layer on top of the medium within seconds of adding the reagents indicated a positive reaction while no colour change indicated a negative reaction.

3.6 Identification and Characterization of Fungal Isolates

Fungal isolates were identified by microscopic and macroscopic techniques as described by Onyeze *et al.* (2013)**.** This is done by using wet mount technique to view the fungi

microscopically and also checking the growth pattern, pigmentation and presence of septa for the macroscopy of the fungi. The fungal isolates were identified by comparing their characteristics with those of known taxa using the schemes of Salako (1994).

3.7 Aseptic Preparation of Bean Cake in the Laboratory

Bean cake was prepared aseptically following the hazard analysis and critical control points (HACCP) standard. About 200grams of Beans obtained from Kure market, Minna, was washed to remove dirt and coat. Washed pepper and onions were added to it and blended using a sterile blender. The blended bean paste was transferred into a clean bowl and stirred in a continuous circular pattern, salt was added (to taste). Oil of about 3 inches deep was heated and the paste was then scooped with a table spoon into the hot oil and was allowed to fry till light brown, it was then flipped to enable the top to fry too. Clean basket was used to collect the bean cake and allowed to drain, which was later transferred into a sterile container. Isolation of microorganisms from the aseptically prepared bean cake was carried out. Figure I shows the flowchart for the preparation of fried bean cake (Akara).

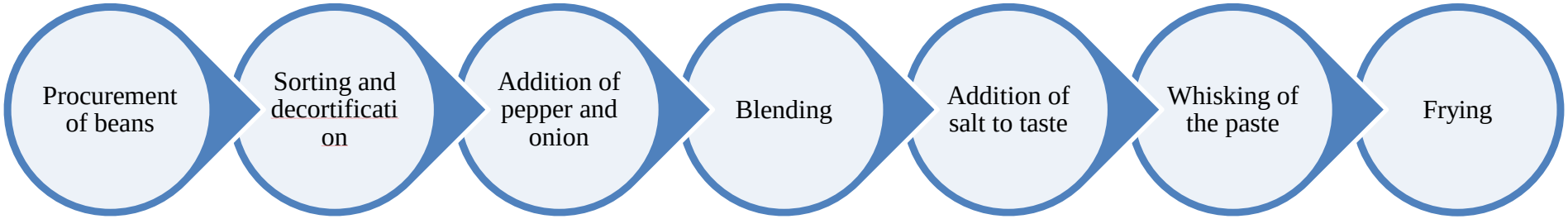

Fig I: Flowchart for the preparation of fried Bean cake

3.8 Molecular Identification of Isolates

3.8.1 DNA extraction

DNA was extracted using the protocol stated by Tamura and Nei (1993). Briefly, Single colonies grown on medium were transferred to 1.5 ml of liquid medium and cultures were grown on a shaker for 48 h at 28 ºC. After this period, cultures were centrifuged at 4600 revolution per minute (rpm) for 5 minutes. The resulting pellets were re-suspended in 520-µl of TE buffer (10 mMTrisHCl, 1mM EDTA, pH 8.0). Fifteen microliters of 20% SDS and 3µl of Proteinase K (20 mg/ml) were then added. The mixture was incubated for 1 hour at 37 ºC, then 100µl of 5 M NaCl and 80-µL of a 10% CTAB solution in 0.7 M NaCl were added and votexed. The suspension was incubated for 10 min at 65 ºC and kept on ice for 15 minutes. An equal volume of chloroform: isoamyl alcohol (24:1) was added, followed by incubation on ice for 5 minutes and centrifugated at 7200rpm for 20 minutes. The aqueous phase was then transferred into a new tube and isopropanol (1: 0.6) was added and DNA precipitated at –20 ºC for 16 h. The DNA was collected by centrifugation at 13000rpm for 10 minutes, washed with 500-µl of 70% ethanol, air dried at room temperature for approximately three hours and finally dissolved in 50-µl of TE buffer.

3.8.2 Polymerase chain reaction

The PCR sequencing preparation cocktail consisted of 10µl of 5x GoTaq colourless reaction, 3µl of 25mM MgCl2, 1-µl of 10 mM of dNTPs mix, 1µl of 10 pmol each 27F 5’ AGA GTT TGA TCM TGG CTC AG3’ and 1525R, 5′AAGGAGGTGATCCAGCC3′ primers and 0.3units of Taq DNA polymerase (Promega, USA) made up to 42-µl with sterile distilled water 8µl DNA template. The PCR was carried out in a GeneAmp 9700 PCR System Thermalcycler (Applied Biosystem Inc., USA) with a PCR profile consisting of an initial denaturation at 94°C

for 5 min; followed by 30 cycles consisting of 94°C for 30 s, 50°C for 60s and 72°C for 1 minute 30 seconds; and a final termination at 72°C for 10 mins. and chilled at 4^{o}C.GEL (2,3)

3.8.3 Integrity of amplified DNA

The integrity of the amplified 1.5Mb gene fragment was checked on a 1% Agarose gel done to confirm amplification. The buffer (1XTAE buffer) was prepared and subsequently used to prepare 1.5% agarose gel. The suspension was boiled in a microwave for 5 min. The molten agarose was allowed to cool to 60°C and stained with 3-µl of 0.5 g/ml ethidium bromide (which absorbed invisible UV light and transmits the energy as visible orange light). A comb was inserted into the slots of the casting tray and the molten agarose was poured into the tray. The gel was allowed to solidify for 20 min to form the wells. The 1XTAE buffer was poured into the gel tank to barely submerge the gel. Two microliter (2-µl) of 10X blue gel loading dye (which gives colour and density to the samples to make it easy to load into the wells and monitor the progress of the gel) was added to 4-µl of each PCR product and loaded into the wells after the 100bp DNA ladder was loaded into well 1. The gel was electrophoresed at 120V for 45 min visualized by ultraviolet trans-illumination and photographed. The sizes of the PCR products were estimated by comparison with the mobility of a 100bp molecular weight ladder that was ran alongside experimental samples in the gel.

3.8.4 Purification of amplified product

The amplified fragments were ethanol purified in order to remove the PCR reagents. Briefly, 7.6-µl of Sodium acetate 3M and 240-µl of 95% ethanol were added to about 40-µl PCR amplified product in a new sterile 1.5µl tube eppendorf, mixed thoroughly by vortexing and kept at 20°C for at least 30 min. Centrifugation for 10 min at 13000 g and 4°C followed by removal of supernatant (invert tube on trash once) after which the pellet were washed by adding 150 µl of 70% ethanol and homogenized then centrifuged for 15 min at 7500rpm and 4°C. All

supernatant was removed (by inverting tube on trash) and inverting tube on paper tissue and allowed to dry in the fume hood at room temperature for 10-15 min. Then re-suspended with 20-µl of sterile distilled water and kept at 20^{o}C prior to sequencing. The purified fragment was checked on a 1.5% Agarose gel ran on a voltage of 110V for about 1h as previous, to confirm the presence of the purified and quantified product using a nano drop of model 2000 from thermo scientific.

3.8.5 Sequencing of isolates

The amplified fragments were sequenced using a Genetic Analyzer 3130xl sequencer from Applied Biosystems using manufacturers' manual while the sequencing kit used was that of BigDye terminator v3.1 cycle sequencing kit. Bio Edit software and MEGA 6 were used for all genetic analysis.

3.9 Antimicrobial Susceptibility Testing

The isolated organisms were tested for their sensitivity/ resistivity to antibiotics using the disk diffusion method of Bassiri (2020). A paper disk impregnated with antibiotics was applied to the surfaces of agar plates containing the isolates to be tested. The plates were incubated at 37^{0}C for 24h. After the incubation, zones of inhibition was measured in millimeter. The antibiotics used were: augmentin (AUG), ofloxacin (OFL), ceftriazone (CRO), gentamycin (GEN), cotrimoxazole (COT), ciprofloxacin (CPX), cloxacillin (CXL), erythromycin (ERY), clindamycin (CLD), cephaplexin (CPX), pefloxacin (PFX) for the bacterial isolates.

3.10 Antifungal Susceptibility Testing

The isolated fungi were tested for their sensitivity using the disk diffusion method of Bassiri (2020). A paper disk containing ketoconazole, fluconazole, nystatin, enilconazole and voriconazole was applied to the surface of agar plate containing the isolates to be tested. The

plates were incubated at 25^{o}C for 48 hours. After the incubation, zones of inhibition were measured in millimeter.

3.11 Identification of Hazards and Critical Control points in the Preparation of fried bean cake

Identification of hazards and critical control point was carried out through visitations of the 14 sale points that were designated for the study. Production and selling of the akara were monitored. Several other parameters were observed, among which are: the hygienic condition of the environment, personal hygiene of the producers and sources of water used for the production, packaging and storage of akara. Samples of paste and water used in the production of akara were obtained and also analysed. All these were done to identify the likely sources of contamination in the production and marketing chains of akara. Samples of paste and water used for production were treated as described in the total microbial load assay.

3.12 Determination of Frequency of Occurrence of Microbial Isolates from Bean Cake

The frequency of occurrence of the isolates was determined by counting the number of occurrence of a particular organism compared to the total organisms isolated from all the bean cake samples.

3.13 Data Analysis

Results were expressed as the mean values ± standard error of mean (SEM) by measuring three independent replicates. Analysis of variance (ANOVA) using one-way was done and Duncan's test was performed to test the significance difference between means values obtained among the treatments at the 5% level of significance using SPSS software (version 21, IBM SPSS). Differences were considered significant at $p<0.05$.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 RESULTS

4.1.1 Bacterial load of Bean Cakes Sold in Minna Metropolis

The effects of the different collection methods (namely, bare hand + nylon, bare hand + clean container, fork + clean container) on the bacterial counts of bean cakes sold in Minna metropolis is shown in table 4.1. The bacterial counts of bean cakes sold in Tunga market were not significantly (P>0.05) different from each other. At Bosso, the bacterial load of the bean cakes collected with fork + clean container was the least while the bacterial counts of bean cakes collected with bare hand + nylon and bare hand + clean container were not significantly (P>0.05) different from each other.

In Dutsen kura, there was no bacterial growth in the bean cakes collected with fork + clean container while the bean cakes collected with bare hand + nylon and bare hand + clean container were not significantly (P>0.05) different from each other but higher than that collected with fork + clean container. The bacterial counts of bean cakes against the mode of collection for Mobil were not significantly (P>0.05) different from each other but they were higher than the control.

At Kpakungu, the bacterial counts of the bean cakes collected with bare hand + nylon and bare hand + clean container were not significantly (P>0.05) different from each other but they were higher than the bacterial counts of bean cakes collected with fork + clean container. At F-layout, the bacterial counts of the bean cakes collected with bare hand + nylon and bare hand + clean container were not significantly different at (P > 0.05) from each other, but higher

than the counts for bean cakes collected with fork + clean containers. The bacterial counts of bean cakes collected from Maitumbi were significantly ($P < 0.05$) different from each other. The bean cakes collected with fork + clean containers had the lowest bacterial counts, followed by the bean cakes collected with bare hand + clean container while the cakes collected with bare hand + nylon had the highest bacterial count.

The bacterial load of bean cakes collected with bare hand + nylon from Tunga and Mobil were the least when compared with the other areas and the control while that of Bosso, Dutsen Kura, Kpakungu and F-layout were not significantly ($P>0.05$) different from each other but higher than that of Tunga and Mobil. The microbial load of bean cakes collected with bare hand + nylon was highest in Maitumbi when compared with that of other areas and the control. The bacterial counts of bean cakes for Kpakungu were significantly ($P < 0.05$) different from each other. The bacterial counts of the bean cakes collected with bare hand + nylon and bare hand + clean container were not significantly($P > 0.05$) different from each other, but higher than the bean cakes collected with fork + clean containers.

The bacterial load of bean cakes collected with bare hand + clean container in Tunga and Mobil were the least when compared with other areas and the control while that of Bosso, Dutsen kura, Kpakungu F-layout and Maitumbi were not significantly ($P>0.05$) different from each other but significantly ($P<0.05$) higher than that of Tunga, Mobil and the control.

There was no bacterial growth in bean cakes collected with fork + clean containers from Dutsen kura when compared with other areas. The bacterial load in bean cakes collected from Dutsen kura and the control are comparable to each other. Generally, bean cakes collected with fork + clean container had the least bacterial load when compared with other mode of collection.

Table 4.1 Bacterial load of Bean Cakes Sold in Minna Metropolis, Nigeria

Mode of Collection	Bacterial Count (cfu/g) x10^5 of bean cakes from various sale points							
	Tunga	**Bosso**	**Dutsen Kura**	**Mobil**	**Kpakungu**	**F-Layout**	**Maitumbi**	**Control (L)**
Bare Hand + Nylon	1.75±0.15^a	2.54±0.13^b	1.75±0.19^b	1.55±0.18^a	1.83±0.17^b	2.58±0.10^b	2.46±0.17^c	0.00±0.00
Bare Hand + Clean Container	1.33±0.16^a	2.16±0.15^b	1.54±0.10^b	1.16±0.14^a	2.75±0.15^b	1.75±0.19^b	1.33±0.17^b	0.00±0.00
Fork + Clean Container	1.70±0.11^a	1.66±0.11^a	0.00±0.00^a	0.42±0.14^a	0.08±0.00^a	0.33±0.12^a	0.25±0.15^a	0.00±0.00

Values are mean ± standard error of mean of triplicate determinations. Values with the same superscript in the same column are not significantly different at $p<0.05$

L: Aseptically prepared bean cake

4.1.2 Coliform counts of bean cakes sold in Minna metropolis

The effects of the different collection methods (namely, bare hand + nylon, bare hand + clean container, fork + clean container) on the coliform counts of bean cakes sold in Minna metropolis is shown in table 4.2. The coliform counts of bean cakes from Tunga, Mobil and F-layout were not significantly ($P>0.05$) different from each other, but higher than the control. There was no coliform growth on bean cakes sold at Bosso and Dutsen kura for all the modes of collection. This result is similar to the control. The coliform counts of bean cakes sold at Mobil for all the modes of collection are not significantly ($P>0.05$) different from each other but higher than the control. The coliform count of bean cake sold at Kpakungu collected with bare hands + nylon and bare hand + clean container are not significantly ($P>0.05$) different from each other but higher than those collected with fork and clean container. The coliform counts of the bean cakes sold at F-layout for all the modes of collection are not significantly different from each other but higher than the control. The coliform counts of bean cakes sold at Maitumbi were significantly ($P<0.05$) different from each other. The bean cake sold with bare hand + nylon had the highest coliform counts while the bean cakes sold with Fork + clean container had the least coliform counts. The coliform counts of bean cakes sold in Maitumbi were significantly ($P<0.05$) higher than the control.

Table 4.2 Coliform Counts of Bean Cakes Sold in Minna Metropolis, Nigeria

Mode of Collection	Coliform Count (cfu/g) x10^5 of bean cakes from various sale points							
	Tunga	**Bosso**	**Dutsen Kura**	**Mobil**	**Kpakungu**	**F-Layout**	**Maitumbi**	**Control(L)**
Bare Hand + Nylon	1.43±0.12[a]	0.00±0.00	0.00±0.00	1.0±0.16[a]	1.25±0.15[b]	1.08±0.17[a]	0.67±0.16[c]	0.00±0.00
Bare Hand + Clean Container	0.95±0.10[a]	0.00±0.00	0.00±0.00	0.42±0.12[a]	1.75±0.11[b]	0.83±0.01[a]	0.46±0.11[b]	0.00±0.00
Fork + Clean Container	0.83±0.22[4a]	0.00±0.00	0.00±0.00	0.33±0.11[a]	0.02±0.02[a]	0.25±0.18[a]	0.01±0.17[a]	0.00±0.00

Values are mean ± standard error of mean of triplicate determinations. Values with the same superscript in the same column are not significantly different at $p<0.05$

L: Aseptically prepared bean cake

4.1.3: Fungal load of bean cakes sold in Minna metropolis

The effect of the different collection methods (namely, bare hand + nylon, bare hand + clean container, fork + clean container) on the fungal counts of bean cakes sold in Minna metropolis is shown in table 4.3. There was no fungal growth on bean cakes sold in Tunga and Bosso. This result is comparable with the control. The fungal counts of bean cakes sold at Dutsen kura were significantly ($P<0.05$) different from each other. The bean cakes collected with fork + clean container did not yield fungal growth while the bean cake sold with bare hand + nylon had the highest fungal count. At Mobil there was no fungal growth on the bean cakes collected with bare hands + clean container and fork + clean container. There was significant fungal growth in the bean cakes collected with bare hand + nylon. At Kpakungu, there was no fungal growth on the bean cakes collected with bare hands + clean containers as well as fork + clean container. There was significant fungal growth on the bean cakes collected with bare hands + nylon. At F-layout, the fungal count of the bean cake collected with bare hand + nylon was significantly ($P<0.05$) different from the bean cakes collected with bare hands + clean container and fork + clean container. There was no fungal growth from the bean cakes sold with bare hands + clean container and fork + clean container.

The fungal counts on bean cakes sold with bare hand + nylon at Dutsen kura, Mobil, Kpakungu, F-layout, and Maitumbi are not significantly different from each other but higher than the control. There was no fungal growth on the bean cakes collected from Tunga and Bosso which are the same with that of the control. There was no fungal growth on the bean cake collected with bare hand + clean container at Tunga, Bosso, Mobil, Kpakungu, F-layout and Maitumbi which is the same with that of the control. The fungal counts of the bean cakes collected with bare hand + clean container at Dutsen kura was significantly ($P<0.05$) higher than the other areas. There was no fungal growth in the bean cakes collected with fork + clean container from all the areas which is similar to that of the control.

4.1.3: Fungal Load of Bean Cakes Sold in Minna Metropolis, Nigeria

Mode of Collection	Fungal Count (cfu/g) x10^3 of bean cakes from various sale points							
	Tunga	**Bosso**	**Dutsen Kura**	**Mobil**	**Kpakungu**	**F-Layout**	**Maitumbi**	**Control(L)**
Bare Hand + Nylon	000±0.00	0.00±0.00	2.00±0.11^b	8.33±0.17^b	2.00±0.11^b	2.33±0.19^b	2.66±0.18^b	0.00±0.00^a
Bare Hand + Clean Container	0.00±0.00	0.00±0.00	0.83±0.11^c	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a
Fork +Clean Container	0.00±0.00	0.00±0.00	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a

Values are mean ± standard error of mean of triplicate determinations. Values with the same superscript in the same column are not significantly different at $p<0.05$

L: Aseptically prepared bean cake

4.1.4 Cultural characteristics of bacterial isolates

The cultural characteristics of the bacterial isolates obtained on both nutrient and MacConkey agar are shown in table 4.4. The organisms isolated include *Bacillus subtilis, Micrococcus roseus, Staphylococcus aureus*, *Klebsiella pneumoniae* and *Escherichia coli.*

4.1.5 Microscopic, morphological and biochemical characteristics of bacteria isolated from Bean cakes

The microscopic, morphological and biochemical characteristics of the bacterial isolates are shown in table 4.5. *Bacillus subtilis, Klebsiella pneumoniae, Staphylococcus aureus*, and *Escherichia coli* were oxidase negative while *Micrococcus roseus* was oxidase positive. All the test isolates were catalase positive. *Bacillus subtilis, S. aureus* and *M. roseus* were Gram-positive while *K. pneumoniae* and *E. coli* were Gram-negative. *Bacillus subtilis, K. pneumoniae, E, coli* and *M. roseus* were coagulase negative while *S. aureus* was coagulase positive. *Bacillus subtilis, K. pneumoniae, S. aureus* and *M. roseus* were indole negative while *E. coli* was indole positive. *Bacillus subtilis, K. pneumoniae* and *M. roseus* were methyl red negative while *S. aureus* and *E. coli* were methyl red positive. *Bacillus subtilis, K. pneumoniae* and *S. aureus* were positive to both Voges-Proskauer and citrate utilization test while *E. coli* and *M. roseus* were negative to the test. *Bacillus subtilis*, and *E. coli* were negative to urease test while *K. pneumoniae, S. aureus* and *M. roseus* were urease positive. *Bacillus subtilis, K. pneumonia*e, *S. aureus* and *E. coli* did not produce hydrogen sulphide gas while *M. roseus* produced the gas.

4.1.6 Cultural and morphological characteristics of fungal isolates from Bean cake

One genera of mould namely, *Aspergillus* was isolated from the bean cake on the basis of its colonial morphology, and microscopic characteristics. The identity of the fungus was cross matched with known taxa. The cultural and morphological characteristics of fungal isolated from bean cakes is shown in table 4.6. Black colonies were isolated on saboraud dextrose agar. And on microscopic observation, rough walled septate hyphae, dark brown to black colour were seen.

4.1.7 Frequency of Occurrence of the Microorganisms Isolated from Bean Cake sold in Minna Metropolis

Staphylococcus aureus was the most frequently isolated organisms from all the bean cakes while *Aspergillus niger* was the least. The frequency of occurrence of the microorganisms isolated from bean cake sold in Minna Metropolis is shown in table 4.7.

Table 4.4: Cultural Characteristics of the Bacterial Isolates

Nutrient Agar	MacConkey Agar	Suspected organisms
Large, slimy colony no pigmentation and very mucoid	Large, slimy colony; pink center and very mucoid	*Klebsiella pneumonia*
Circular small colonies with yellow pigment	ND	*Staphylococcus aureus*
Large, irregular spreading colony		*Bacillus subtilis*
Circular, entire, convex with red pigmentation	ND	*Micrococcus roseus*
Large, thick, greyish white, smooth and translucent disc	Large, doughnut shaped and dark pink colonies	*Escherichia coli*

ND – not determined

Table 4.5: Microscopical, Morphological and Biochemical Characteristics of the Bacterial Isolates

GR	SHAPE	COG	OX	CT	IN	MR	VP	CI	UR	H_SS	Suspected organisms
+	Rod	_	_	+	_	_	+	+	_	_	*Bacillus subtilis*
_	Rod	_	_	+	_	_	+	+	+	_	*Klebsiella pneumoniae*
+	Cocci	+	_	+	_	+	+	+	+	_	*Staphylococcus aureus*
_	Rod	_	_	+	+	+	_	_	_	+	*Escherichia coli*
+	Cocci	_	+	+	_	_	_	_	+	-	*Micrococcus roseus*

GR: Gram's reaction, COG: Coagulase test, OX: Oxidase test, CT: Catalase test, MR: Methyl red test, IN: Indole test, UR: Urease test, H_2S: Hydrogen Sulphide Production test, L: Lactose sugar fermentation test, S: Sucrose sugar fermentation test, G: Glucose sugar fermentation test, VP: Voges Proskauer test, CI: Citrate utilization test, +: Positive, -: Negative

Table 4.6: Cultural and Morphological Characteristics of Fungal Isolates from Bean Cake

Cultural characteristics	Microscopic characteristics	Organisms suspected
Black colony on SDA	Septate hyphae, dark brown to black and rough walled	*Aspergillus niger*

Table 4.7: Frequency of Occurrence of Bacterial isolates of Bean Cakes sold in Minna Metropolis

Microorganisms	Number	% Frequency of occurrence
Staphylococcus aureus	60	23.08
Escherichia coli	53	20.38
Bacillus subtilis	45	17.31
Micrococcus roseus	42	16.15
Bacillus megatarium	29	11.15
Klebsiella pneumonia	25	9.62
Aspergillus niger	6	2.31

4.1.8 Molecular Identities of the Isolates

The molecular characterization using 16rRNA confirmed the isolates as *Bacillus subtilis, Klebsiella pneumoniae, Staphylococcus aureus, Escherichia coli, Micrococcus roseus* and *Aspergillus niger.* Table 4.1.8 shows the accession number of the isolates. The summary of molecular characteristics of the tests organisms are presented in plates III and IV respectively.

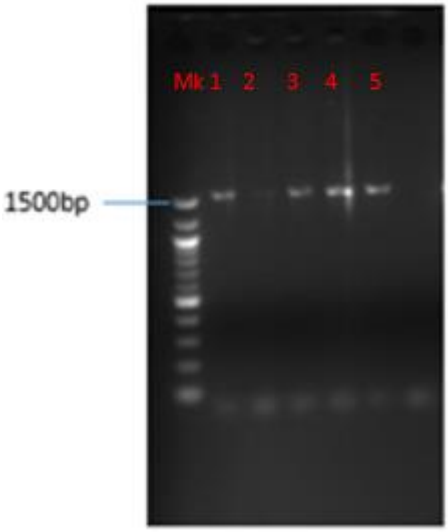

Plate III: Gel electrophlorograph showing the positive amplification of bacterial isolates using the 16S universal primers. A band size of about 1500bp indicated a positive amplification

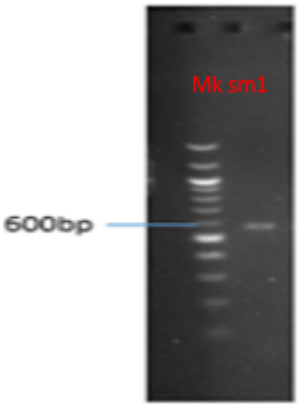

Plate IV: Gel electrophlorograph showing the positive amplification of sabr6 (fungi) isolate using the ITS universal primers. A band size of about 550bp indicated a positive amplification

Table 4.1.8: Accession Number of the Isolates.

Scientific Name	Max Score	Total Score	Query Cover	Identity %	Accession Number
Bacillus subtilis	2695	2695	100%	100.00%	MZ185316
Klebsiella pneumonia	2656	2656	100%	99.79%	MZ185317
Staphylococcus aureus	2695	2695	100%	100.00%	MZ185318
Escherichia coli	2543	2543	100%	100.00%	MZ185319
Micrococcus roseus	2193	2193	99%	99.50%	MZ185320
Aspergillus niger	1037	1037	98%	100.00%	MZ171448

4.1.9 Antimicrobial Susceptibility Profile of the Bacteria Isolated from Bean Cakes sold in Minna Metropolis

The susceptibility pattern of the bacteria isolated from Bean Cakes sold in Minna metropolis is shown in table 4.9. *Staphylococcus aureus* was resistant to ciprofloxacin, less susceptible to ceftriazone, gentamycin, pefloxacin and clindamycin but most susceptible to erythromycin, cloxacillin, and clotrimazole, followed by augmentin and ofloxacin. *Escherichia coli* was most susceptible to ciprofloxacin, ofloxacin, augmentin and cotrimazole followed by ceftriazone and clindamycin and least susceptible to gentamycin, pefloxacin, cloxacillin and erythromycin. *Klebsiella pneumoniae* was most susceptible to augmentin and ciprofloxacin followed by gentamycin, ceftriazone and cotrimazole but least susceptible to ofloxacin, pefloxacin, cloxacillin, erythromycin and clindamycin. *Micrococcus roseus* was most susceptible to

gentamycin, cloxacillin and clindamycin followed by augmentin and ofloxacin but least susceptible to ciprofloxacin, pefloxacin and erythromycin and it was resistant to ceftriazone and cotrimazole. *Bacillus subtilis* was most susceptible to clindamycin followed by augmentin and cotrimazole but least susceptible to ceftriazone, gentamycin, pefloxacin, cloxacillin and erythromycin but resistant to ciprofloxacin and ofloxacin.

4.1.10 Antifungal susceptibility testing

The susceptibility of the fungal isolates to antifungi agents is as shown in Table 4.10. *Aspergillus niger* was most susceptible to enilconazole, voriconazole followed by ketoconazole and least susceptible to nystatin but resistant to fluconazole.

Table 4.9 Antimicrobial Susceptibility profile of the bacteria isolated from bean cake sold in Minna Metropolis, Nigeria

Isolates	Zone of Inhibition in Diameter (mm) for Antimicrobial Agents									
	CPX	**CRO**	**GEN**	**OFL**	**AUG**	**COT**	**PFX**	**CXC**	**ERY**	**CLD**
Escherichia coli	20.00 ± 2^{c}	16.00 ± 0^{b}	10.00 ± 4^{a}	18.00 ± 3^{c}	20.00 ± 1^{c}	17.00 ± 0^{b}	10.00 ± 1^{a}	5.00 ± 0^{a}	9.00 ± 2^{a}	12.00 ± 2^{b}
Klebsiella pneumoniae	19.00 ± 0^{c}	14.00 ± 4^{b}	15.00 ± 0^{b}	10.00 ± 0^{a}	25.00 ± 4^{c}	16.00 ± 3^{b}	10.00 ± 0^{a}	5.00 ± 2^{a}	5.00 ± 1^{a}	10.00 ± 0^{a}
Staphyloccus aureus	0.00 ± 0^{a}	7.00 ± 1^{b}	8.00 ± 1^{b}	14.00 ± 2^{c}	15.00 ± 0^{c}	17.00 ± 6^{c}	7.50 ± 0.5^{b}	20.00 ± 5^{d}	27.00 ± 2^{d}	6.50 ± 0.5^{b}
Micrococcus roseus	10.00 ± 3^{b}	0.00 ± 0^{a}	25.00 ± 2^{d}	13.00 ± 2^{bc}	15.50 ± 2.5^{c}	0.00 ± 0^{a}	10.00 ± 5^{b}	20.00 ± 0^{d}	6.00 ± 1^{b}	25.00 ± 2^{d}
Bacillus subtilis	0.00 ± 0^{a}	6.50 ± 0.5^{b}	7.00 ± 0^{b}	0.00 ± 0^{a}	15.00 ± 0^{c}	15.00 ± 0^{c}	10.00 ± 0^{b}	6.00 ± 0^{b}	8.00 ± 0^{b}	18.00 ± 0^{c}

Values are mean of zones of inhibition ± standard error of mean of triplicate determinations. Values with the same superscript letters in the same column are not significantly different at $p>0.05$

CPX: Ciprofloxacin CRO: Ceftriazone, GEN: Gentamycin, OFL: Ofloxacin, AUG: Augmentin, COT: Cotrimoxazole, PFX: Pefloxacin, CXC: Cloxacillin, ERY: Erythromycin, CLD: Clindamycin

Table 4.10 Antifungal Susceptibility Profile of *Aspergillus niger* Isolated from Bean Cake sold in Minna Metropolis, Nigeria

	Zone of Inhibition in Diameter (mm) for Antifungal Agents				
Isolate	**Nystatin**	**Fluconazole**	**Ketoconazole**	**Enilconazole**	**Voriconazole**
Aspergillus niger	11.00±2.00	0.00±0.00	13.50±1.00	16.00±0.00	14.50±1.00

Values are mean of zones of inhibition ± standard error of mean of triplicate determinations. Values with the same superscript letters in the same column are not significantly different at $p>0.05$

4.1.11 Sources of Hazards associated with Bean Cake

The bacterial load and coliform counts of water used for bean cake production in Minna metropolisis shown in table 4.11. The bacterial counts of water collected from Bosso was the highest while that of Mobil was the least. The bacterial counts of water collected from Bosso and Kpakungu are not significantly (P>0.05) different from each other but higher than the control. The bacterial counts of water collected from Tunga and F-layout are not significantly (P>0.05) different from each other but higher than the control. The coliform counts of water collected from F-layout was the highest while that of Maitumbi was the least. The coliform counts of water collected from Maitumbi, Mobil, Kpakungu, Dutsen kura, and Bosso are not significantly (P>0.05) different from each other but higher than the control.

The bacterial load and coliform counts of bean paste in Minna metropolis is shown in table 4.1.11b. The bacterial counts of bean paste collected from Tunga was the highest while that of Mobil was the least. The bacterial counts of bean paste collected from Maitumbi, Kpakungu, Dutsen kura and Bosso was not significantly (P>0.05) different from each other but higher than the control. The coliform counts of bean paste collected from Tunga was the highest while that of Mobil, Dutsen kura and Bosso was the least.

Table 4.11 Microbial Load of Water used for Bean Cake Production in Minna Metropolis, Nigeria

Sale Points	Bacterial Counts (cfu/g) x 10^5	
	Bacterial Count	**Coliform Count**
Tunga	3.50 ± 0.14^{c}	1.75 ± 0.15^{bc}
Bosso	5.00 ± 1.00^{d}	1.25 ± 0.15^{b}
Dutsen Kura	3.00 ± 1.00^{bc}	1.25 ± 0.15^{b}
Mobil	2.50 ± 1.50^{b}	1.40 ± 0.10^{b}
Kpakungu	4.50 ± 1.50^{d}	1.50 ± 0.12^{b}
F-Layout	3.50 ± 1.50^{c}	2.00 ± 0.12^{c}
Maitumbi	6.50 ± 0.10^{e}	1.10 ± 0.10^{b}
Control	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Values are mean ± standard error of mean of triplicate determinations. Values with the same superscript letters in the same column are not significantly different at $p<0.05$

Table 4.11b Microbial Load of Bean Paste used for Bean Cake Production in Minna Metropolis, Nigeria

	Bacterial Counts (cfu/g) x 10^5	
Sale Points	**Bacterial Count**	**Coliform Count**
Tunga	3.00 ± 0.10^{d}	2.70 ± 0.10^{d}
Mobil	1.25 ± 0.15^{b}	1.00 ± 0.15^{b}
Maitumbi	1.95 ± 1.50^{c}	1.25 ± 0.10^{b}
Kpakungu	2.00 ± 0.10^{c}	1.5 ± 0.10^{c}
Flayout	2.95 ± 0.01^{d}	2.50 ± 0.01^{d}
Dutsen kura	1.75 ± 0.15^{c}	1.00 ± 0.10^{b}
Bosso	1.75 ± 0.15^{c}	1.00 ± 0.10^{b}
Control	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Values are mean ± standard error of mean of triplicate determinations. Values with the same superscript letters in the same column are not significantly different at $p<0.0$

4.2 Discussion

In the present study, a variety of microorganisms had been isolated from bean cake based on different collection methods (namely, bare hand + nylon, bare hand + clean container, fork + clean container) Generally, bean cakes collected with Bare hands + nylon had the highest microbial load while those collected with fork + clean container had the least microbial load. This could be due to certain unhygienic practices of the producer. Examples of such unhygienic practices include: blowing air into the nylon with the mouth which may introduce oral contaminants into the nylon, using bare hands to pick bean cakes which might introduce contaminants into the bean cakes. This is similar to the findings of Lateef *et al.* (2016), where it was observed that contamination of bean cake usually occur at the post processing stage.

Coliforms were detected in the bean cakes sold in Minna metropolis. This indicate that the raw materials used for making Akara were contaminated with fecal matter. This is in agreement with the study of Shih-Chun *et al.* (2017) who observed that fecal matters might be responsible for coliform growth in a sample. The coliform counts of bean cakes from all the sale points ranged from 0±0.0 cfu/g in Bosso and Dutsen kura to $1.75x10^5 \pm 1.09x10^4$ cfu/g in Kpakungu. There was no coliform growth on the aseptically prepared bean cakes and that of those from Bosso and Dutsen kura.

In this study the following organisms were isolated: *Bacillus subtilis, Micrococcus roseus, Staphylococcus aureus, klebsiella pneumoniae, Escherichia coli* and *Aspergillus niger. Staphylococcus aureus* had the highest frequency of occurrence while *Aspergillus niger* had the least. This could be due to the fact that *S. aureus* are widely distributed in the environment and occur on the skin and nostrils of humans (Adele *et al.,* 2018)**.** It is therefore possible that these organisms can be transferred from these sources to the bean cakes during processing.

Aspergillus niger was also isolated. These organisms are widely distributed in the environment and produced spores which are heat resistant and allow them to proliferate in the Akara. Alexandra *et al.* (2021) reported that *Aspergillus* species infects peanuts and other foods producing aflatoxins, a potent carcinogen. This is similar to the findings of Alum *et al.* (2016) where species of *bacillus, Staphylococcus* and *Aspergillus* were isolated from bean cake respectively. The findings are also similar to those of the reports for the occurrence of these organisms in a wide variety of food where they cause food infection, poisoning and intoxication (Adele *et al.,* 2018**).** The isolation of these microbes from bean cakes is worrisome because these organisms are potential pathogens of man capable of causing a variety of diseases.

Generally in this study, the bean cakes collected from the sale points had high microbial growth while the aseptically prepared bean cakes yielded no growth. This could be due to the mode of handling of the bean cakes and the level of hygiene of the handlers and the environment. This is in line with the findings of Toledo *et al.* (2018) who observed that aseptic processing can eliminate spoilage microorganisms.

The molecular identification of the isolates confirmed the isolates as *B. subtilis, M. roseus, S. aureus, K. pneumoniae, E. coli* and *A. niger.* This is similar to the findings of Mpinda *et al.* (2018). Who identified these organisms from street -vended ready -to -eat meat
In this study, the antimicrobial susceptibility profile shows that *Staphylococcus aureus, K. pneumoniae* and *M. roseus* were susceptible to 45.45% of the antibiotics used while *E. coli* was susceptible to 31.98 %, *B. subtilis* was susceptible to 27.27 % and *Aspergillus niger* was susceptible to 60 % of the antifungal agents used.

The hazards and critical control points in the production of bean cake are at the bean paste and water used for bean cake production. The microbial load was high at milling stage and in water used for mixing. This indicates confluent growth of organisms in the production process. This

could be due to the fact that the milling machine used and water used contained some microbial contaminants. This is in agreement with the study of Alum *et al.* (2016) who also observed same.

The milling stage and water used for mixing the paste should be proper monitored during the production of Akara. Therefore, these stages are the critical control points (CCP) which are locations, steps or procedure at which some degree of control should be exercised over a microbial hazard. The risk or hazard can be prevented, eliminated or reduced to acceptable levels (Lateef *et al.,* 2010). Loss of control at CCP could result to unacceptable risk to the consumers or products.

The sale points in this study, were not hygienic. This could be as a result of lack of formal education on food processing. Their main sources of water were well, tap and water vendors and their water storage plan was also not hygienic. The type of packaging method used for the sale of bean cake is hand picking into nylon. All these could also be as a result of lack of formal education on food processing. This is in agreement with Lateef *et al.* (2016), where it was observed that lack of formal education on food processing could affect the personal and environmental hygiene of the producer and sale points.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Aspergilus niger, Bacillus subtilis, Micrococcus roseus, Staphylococcus aureus, Klebsiella pneumoniae and *Escherichia coli* were isolated and characterized from bean cake sold in Minna metropolis. The microbial load of the bean cake samples collected were higher than the aseptically produced one. *Staphylococcus aureus, Klebsiella pneumoniae* and *Micrococcus roseus* were susceptible to 45.45 % of the antibiotics examined. The study revealed the critical control points of the production of Akara at bean paste production (milling) and water used for mixing. These are points where some degree of control can be exercised over the microbial hazard to prevent, eliminate or reduce the microbial level to acceptable limits. The isolation of these organisms is a serious public health concern. Their presence is associated with poor environmental hygiene.

5.2 Recommendations

Based on the findings of this research, it is recommended that:

i. thorough washing and mixing of the bean cakes with clean water should be undertaken to reduce the level of microbial contamination.
ii. Aseptic techniques are essential in the sales point.
iii. Contamination of the bean cakes by bare hands can be reduced by hand washing and good personal hygiene. Avoid insect contamination by covering the cakes after frying.

REFERENCES

Adele, S., Fabienne, B., Jean-louis, M, Jean-louis, R. & Olivier, B. (2018). Staphylococcus aureus nasal colonization: an update on mechanisms, Epidemiology, Risk Factors and Subsequent infections. *Frontiers in Microbiology*, 9, 2419-2434.

Ajibola, F. C. & Adelekan, F. (2017). Storage Stability of Deep-fried Cowpea Products (AKARA) Incorporated with Soy-flour and Aframomum danielli. *British Journal of Applied Science & Technology*, 8(2), 204-212.

Akajiaku, L. O., Nwosu, J. N., Odimegwu, E. N., Alagboso, S. O. & Uzoechi, J. C. (2014). Influence of sprouted pigean pea (Cajan cajan) flour inclusion on sensory quality of moi-moi. *The International Journal and Technology*, 2(12), 122-129.

Alexander, S. Hana, V. & Nosalj, S. (2021). *Aspergillus niger*: Environmental Isolates and their specific diversity through Metabolite profiling. *Journal of frontiers in microbiology*, 12(6), 58-65.

Alum, E. A., Urom, S. M., Chukwu, B. & Chukwu M. A. (2016). Microbiological Contamination of Food: The Mechanisms, Impacts and Prevention. *International Journal of Scientific and Technology Research*, 5(03), 3-7.

Appiah, F., Asibuo, J. & Kumah, P. (2011). Physicochemical and functional properties of bean flours of three cowpea (*Vigna unguiculata* L. Walp) varieties in Ghana. *African Journal of Food Science*, 5(2), 100–104.

Aviara, N. A., Olarinde, M. O., Nasirudeen, A. R., Lasisi, L. A., Balogun, S. O., & Ogunsola, F. O. (2018). Empirical analysis of traditional akara frying process. *Arid zone Journal of Engineering, Technology & Environment,* 14(4), 225-236.

Bassiri, E. A (2020). Antimicrobial susceptibility Testing. Retrieved from https://www.sas.upenn.edu. 18th July 2021.

China, M., Nua, J., Deedam, P. M. & Christabel, P. (2019). Proximate composition and sensory assessment of beans pudding prepared using two different cooking methods. *Research Journal of Food Science and Nutrition*, 4(2), 58-64.

Cowpea research at the International Institute of Tropical Agriculture (IITA) Retrieved from https://www.iita.org/cowpea. 27th June 2021.

Eke-Ejiofor, J., & Kporna, J. D. (2019). Nutrient and sensory evaluation of cowpea-Acha flour blends in pudding production. *Food Science and Nutrition Technology*, 4(1), 1-11.

Gonçalves, A., Goufo, P., Barros, A., Domínguez-Perles, R., Trindade, H. R., Eduardo, A. S., Ferreira, L. & Rodrigues, M. (2016). Cowpea (Vigna unguiculata L. Walp), a renewed Multi-purpose crop for a more sustainable agri-food system: nutritional

advantages and constraints. *Journal of the Science of Food and Agriculture.* 96, (9): 2941–2951.

Hamid, S., Muzaffar, S., Wani, I. A., Masoodi, F. A. & Bhat, M. M. (2016). "Physical and cooking characteristics of two cowpea cultivars grown in temperate Indian climate. *Journal of the Saudi Society of Agricultural Sciences,* 15 (2): 127–134.

Kumar, S., Stecher, G., Li, M., Knyaz, C. & Tamura, K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Journal of Molecular Biology and Evolution*, 35, 1547-1549.

Lateef, T. E., Davies, A., Adelekan, I. A., Adelere, A. A. & Fadahunsi, A. H. (2016). Akara Ogbomoso: Microbiological Examination and Identification of Hazards and Critical Control Points. *International Journal of Food Science and Technology,* 16,386-389.

Moutaleb, O. H., Amadou, I. & Amza, T. (2017). Physico-functional and sensory properties of cowpea flour-based recipes (akara) enriched with sweet potato. *Journal on Nutritional Health Food Engineering,*7(4), 325–330.

Mpinda, E. T., Ngoma L., Modupeade, C. A. & Mlunda M. (2018). Molecular Characterization and Antibiotic Resistance of Foodborne Pathogens in Street- Vended ready -to -eat meat sold in South Africa. *Journal of Food Protection*, 81(12), 1963-1972.

Olu, M., Rotimi, A. & Emmanuel, A. A. (2017). Development of spiced instant moi-moi produced from pre-cooked cowpea using maize starch as binder. *International Journal of Food Science and Nutrition Engineering,* 7(4), 75-90.

Olusegun S. O., Jibrin K. M. & Olurotimi K. (2020). A Study of the Application of GIS in the Assessment of the impact of infrastructure on residential property value in Minna. *International Journal of Geography*, 9(3), 45-48.

Onyeze, R. C., Udeh, S. M. C. Akachi, B. O. & Okechukwu, P. C. (2013). Isolation and Characterization of fungi associated with the spoilage of corn (ZEA MAYS). *International Journal of Pharma Medicine and Biological Sciences*, 2, 3-9.

Oumarou, H. M., Issoufou, A. & Tidjani A., (2017) Physico-functional and sensory properties of cowpea flour based recipes (akara) and enriched with sweet potato. *Journal of Nutritional Health & Food Engineering*, 7(4), 85 -91.

Owade, J. O., Abong, G., Okoth, M. & Mwang'ombe, A. W. (2019). A review of the Contribution of Cowpea leaves to food and nutrition security in East Africa. *Journal of Food Science & Nutrition,* 8 (1): 36–47.

Pottorff, M., E., Jeffrey, D., Fatokun, C., Roberts, P. A. & Close, T. J. (2019). Leaf morphology in Cowpea [Vigna unguiculata (L.) Walp]: QTL analysis, physical mapping and identifying a candidate gene using synteny with model legume species. *Journal of Agricultural and Biological Science,* 7, 231-247.

Preetha, S.S & Rita N. (2020). Factors Influencing the Development of Microbes in Food. *Journal of Arts, Science and Humanities*, 7(3), 57–77.

Sagar, A. (2019). Pour plate Technique: Procedure, Advantages and Limitations. Retrieved from https://microbenotes.com

Sakpo, I. O. & Osundahunsi, O. F. (2016). Effect of cowpea enrichment on the physico-chemical, mineral and microbiological properties of maize: cowpea flour blends. *Research Journal of Food Science and Nutrition*, 1(2), 35-41.

Shih‐Chun Y., Chih‐Hung L., Ibrahim A. A. & Jia‐You F. (2017). Current pathogenic *Escherichia coli* foodborne outbreak cases and therapy development. *Journal on Microbiology*, 2 (4), 234-265.

Toledo, R. T. (2018). Fundamentals of Food Process Engineering, Springer International PublishingAG, part of Springer Nature. Retrieved from https://doi.org/10.1007/978-3-319-90098-8-9.

Tamura, K. & Nei, M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Journal of Molecular Biology and Evolution*, 10, 512-526.

Witthöft, C. & Hefni, M (2016). Cowpea. *Encyclopedia of Food and Health*. Oxford: Academic Press. pp. 724–730. ISBN 978-0-12-384953-3. Retrieved from https://doi.org/10.1016%2FB978-0-12-384947-2.00300-7