ASSESSMENT OF BACTERIOLOGICAL AND NUTRITIONAL QUALITIES OF LOCAL CEREAL FOODS PROCESSED IN KPAMBO COMMUNITY, TARABA STATE

BY

MAGAYAKI, Joshua
MTech/SLS/2018/9287

DEPARTMENT OF MICROBIOLOGY
FEDERAL UNIVERSITY OF TECHNOLOGY, MINNA

AUGUST, 2023

ASSESSMENT OF BACTERIOLOGICAL AND NUTRITIONAL QUALITIES OF LOCAL CEREAL FOODS PROCESSED IN KPAMBO COMMUNITY, TARABA STATE

BY

MAGAYAKI, Joshua
MTech/SLS/2018/9287

A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL, FEDERAL UNIVERSITY OF TECHNOLOGY, MINNA, NIGERIA IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE MASTER OF TECHNOLOGY IN FOOD AND INDUSTRIAL MICROBIOLOGY

AUGUST, 2023

ABSTRACT

There has been a rise in the incidence of food borne diseases (FBDs) from ready to eat – foods (RTF) mostly in development countries like Nigeria which is linked to poor sanitary and unhygienic food processing practices. *Ujwumbae* and *iraen* are nutritious and locally processed ready to-eat cereal based foods consumed on daily basis in Kpambo Community of Taraba State, Nigeria. Samples of *ujwumbae* – a guinea corn based porridge and *iraen* – a maize based meal were obtained as freshly prepared locally from food vendors in Kpambo Community, Ussa Local Government Area of Taraba State, Nigeria between the months of April and July, 2021 and screened for bacteriological and nutritional qualities using standard methods. The *ujwumbae* had high total aerobic bacterial counts (TABC) ranged from 1.0 x10^2 – 6.4 x10^4 cfu/mL, total coliform counts (TCC) ranged from 1.4 x10^2 - 7.8 x 10^2 cfu/mL while the *irean* had TABC ranged from 1.3 x 10^6 8.3 x 10^8 cfu/g and TCC 1.0 x 10^3 - 8.0 x 10^5 cfu/g. No total aerobic fungi count (TAFC) were detected in the two cereal foods analyzed. Bacteria identified were *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella oxytoca* and *Klebsiella pneumoniae.* The frequency occurrence of these isolates were: *S. aureus (50%* in *ujwumbae* and 36.39 % *in iraen), E. coli.* (25% in *ujwumbae and* 22.73% in *irean), P. aeruginosa* (10% in *ujwumbae* and 13.64% in *iraen*), *Klebsiella oxytoca* (5% in *ujwumbae* and 18.18% in *irean*) and *Klebsiella pneumoniae* (10% in *ujwumbae* and 9.09% in *irean).* The percentage proximate nutritional contents of the cereal foods analyzed ranged in this order: moisture (93.90 – 96.99%) for *ujwumbae* and 70.90 - 96.44% for *irean*); ash (0.04 – 0.34% for *ujwumbae* and 0.09 – 1.25% for *iraen*), fat (0.40 – 0.54% for *ujwumbae* and 1.55 – 2.36% for *irean*), Protein (3.03 – 4.0% for *ujwumbae* and 4.75 – 6.30% for *irean*); Carbohydrate (0.81 – 29.24% for *ujwumbae* and 9.47 – 20.84% for *irean,* and no fibre was detected for the two food samples analyzed. The bacteria were tested for antibiotics susceptibility using disc diffusion method. The bacterial isolates mostly demonstrated susceptibility to the tested antibiotics in this order: *S. aureus* were susceptible to ciprofloxacin (10mcg) at the ranged of 21 – 35mm, amoxicillin (30mcg) at the range of 13 – 24mm; *E coli* were susceptible to ceftriaxone (30mcg), gentamicin (10mcg), tetracycline (30mcg) at the range of 20 – 30mm; *P. aeruginosa* were susceptible to ceftriaxone (30mcg), gentamicin (10mcg), tetracycline (30mcg) and amoxicillin (30mcg) at the range of 15 – 40mm; *K oxytoca* were susceptible to ceftriaxone (30mcg), levofloxacin (5mcg) and amoxicillin (30mcg) at the range of 15 – 29mm and *K pneumoniae* were susceptible to ceftriaxone (30mcg), gentamicin (10mcg), tetracycline (30mcg), amoxicillin (30mcg) and ofloxacin (5mcg) at the range of 15 – 33mm. The results of this study suggest that the qualities and safety of the locally produced *ujwumbae* and *irean* samples obtained in Kpambo are not good as the bacteria contents were of high number and pathogens were involved. There is the need for proper food processing and handling methods.

TABLE OF CONTENTS

Content	Page
Cover page	i
Title page	ii
Declaration	iii
Certification	iv
Dedication	v
Acknowledgements	vi
Abstract	vii
Table of Contents	viii
List of Tables	xiii
List of Figures	xiv
List of Plates	xv
CHAPTER ONE	
1.0 INTRODUCTION	1
1.1 Background to the Study	1
1.2 Statement of the Research Problem	4
1.3 Aim and Objectives of the Study	5
1.4 Justification for the Study	5
CHAPTER TWO	
2.0 LITERATURE REVIEW	6
2.1 Food Contamination	6
2.1.1 Sources of food contamination	6
2.1.2 Implications of consuming contaminated foods	9
2.2 Contamination and Spoilage of Cereal Foods	10
2.3 Nutritional Qualities of Cereal Foods	11

2.4 Microbiological Qualities of Cereal Foods 14

2.5 Processing of Cereal Foods 14

2.6 Importance of Cereal Foods 15

2.7 Prevention and Control of Cereal Food Contamination and Spoilage 16

2.8 Packaging and Storage of Processed Cereal Foods 17

2.9 Future Prospects of Cereal Foods in Nigeria 18

CHAPTER THREE

3.0 MATERIALS AND METHODS 22

3.1 Study Area 22

3.2 Collection of Samples 25

3.3 Enumeration of Microbial Counts 26

3.3.1 Preparation of serial dilution 26

3.3.2 Determination of bacteria counts and isolation 26

3.3.2.1 *Total coliform / faecal coliform counts* 26

3.3.2.2 *Enumeration of Salmonella and Shigella species* 27

3.4 Characterization and Identification of Bacterial Isolates 28

3.4.1 Morphological identification of isolates 28

3.4.2 Gram staining 28

3.4.3 Biochemical characterization of the isolates 28

3.4.3.1 *Catalase test* 28

3.4.3.2 *Citrate utilization test* 29

3.4.3.3 *Oxidase test* 30

3.4.3.4 *Coagulase test* 30

3.4.3.5 *Indole production test* 30

3.4.3.6 *Motility test (stab culture technique)* 30

3.4.3.7 *Starch hydrolysis test* 31

3.4.3.8 *Sugar utilization and fermentation tests* 31

3.5 Determination of Proximate Nutrient Composition 32

3.5.1. Preparation of food homogenate 32

3.5.2 Determination of moisture content 33

3.5.3 Determination of crude protein 33

3.5.4 Determination of fat 34

3.5.5 Determination of carbohydrate 35

3.5.6 Determination of ash 35

3.5.7 Determination of crude fibre 36

3.6 Determination of Antibiotic Resistance Profile of the Bacterial Isolates 36

3.7 Data Analysis 37

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION 38

4.1. Results 38

4.1.1 Microbial counts and identity in cereal foods 38

4.1.2 Proximate nutritional composition of the locally processed cereal foods obtained from Kpambo 43

4.1.3 Antibiotics resistance profiles of bacterial isolates from *ujwumbae* and *iraen* samples 45

4.2 Discussion of Result 49

4.2.1 Microbial counts and identity in cereal foods 49

4.2.2 Proximate nutritional composition of the locally processed cereal foods obtained from Kpambo 50

4.2.3 Anti-biotic resistance profile of bacterial isolated in locally produced cereal food (*Ujwumbae* and *Irean*) samples from Kpambo 53

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS 54

5.1 Conclusion 54

5.2. Recommendations 54

5.3 Contribution to Knowledge 55

REFERENCES 56

APPENDIX 63

LIST OF TABLES

Table **Pages**

4.1 Total microbial counts of *ujwumbae* samples analysed (cfu/mL) 38

4.2 Total microbial load of *irean* samples analysed (cfu/ml)12q 39

4.3 Characterization and identification of bacteria found in *ujwumbae* and *irean* samples 40

4.4 Distribution of bacteria in *ujwumbae* and *irean* samples 41

4.5 Proximate nutritional contents of *ujwumbae* samples analyzed 43

4.6 Proximate nutritional contents of *irean* samples analyzed 44

4.7 Zone of inhibition (in millimeter) of Gram-positive bacteria isolated from the cereal foods (*ujwumbae* and *iraen*) Samples. 45

4.8 Resistance and susceptible pattern of the Gram negative bacterial isolates from the cereal foods. 46

4.9 Zone of inhibition (in millimeter) of Gram-positive bacteria isolated from the cereal foods (*ujwumbae* and *iraen*) samples. 47

LIST OF FIGURES

Figure **Page**

3.1 Map of Taraba State Showing Ussa Local Government Area (study area) and other Local Government Areas (LGAs) of the State 23

4.1 Distribution of bacteria isolated in local processed cereal Foods (*iraen* and *ujwumbae)* samples. 42

LIST OF PLATES

Plate		Page
I	*Ujumbae* products packaged in plastic bottles	24
II	*Iraen* products wrapped with nylon and placed in a plate	25

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Cereals are the edible seeds or grains of the grass family, Gramineae which are grown in different countries and in greater quantities (Bender and Bender, 1999). On a worldwide basis, they provide more food energy than any other type of crops. They are therefore called staple crops (Idem and Showemimu, 2004) and have a long history of use by humans (Bender and Bender, 1999). In West Africa particularly in Nigeria, Ghana, Burkina Faso, cereal grains are milled and used to produce varieties of foods, which are known by various names in different parts of the continents. Cereal foods are the major sources of energy in Nigeria (Nkama and Gbenyi, 2001). The major cereals crops in Nigeria include Maize, Sorghum, Millet and Rice. The various processed cereal foods in Nigeria are pap (ogi or akamu), *burukutu*- a local alcoholic beverage, *Kunun zaki*, P*ito* and *Ndaleyi* among others (Ismaila *et al.*, 2017; Ukwuru *et al.*, 2018).

The people of Kpambo Community, Ussa Local Government Area of Taraba State often consume locally processed cereal foods. The most common indigenous cereal processed or cooked foods are the *irean* – a maize-based meal and *ujwumbae* – a guinea corn-based porridge. The *irean* and *ujumbae* are the traditional names in *Kuteb* – Taraba State language. The *irean* and *ujumbae* are known as ready to eat foods that are consumed without further processing or preparation. They are traditionally processed, packaged mostly under uncontrolled environment (open environment) in a container and usually consumed immediately or later (Cerna-Cortes *et al.*, 2015). The *irean* and *ujwumbae* constitute a large part of daily diet in the population of Kpambo community. The foods are cheap because the cereals are sourced and cultivated within the community and almost

available throughout the year. These foods are rich in carbohydrate, minerals and a proportion of proteins (Ayo and Okaka, 1998).

The *ujwubae* is simply described as non-alcoholic drink while the *irean* as solid paste (‘swallow’) usually eaten with different varieties of soup like vegetable and ‘draw soup’ (Abduraham and Kolawale, 2006). The *ujwubae* and *irean* can also be processed from other cereal crops like rice, wheat, and millet. Other locally processed cereal foods in the community are Burkutu – a local alcoholic drink and Porridge (pap) which are called *ujwap* (*Kuteb* dialect) and *akamu* respectively. These cereal foods (*irean* and *ujumbae*) are produced in homes for consumption and for sale. The products are also consumed during meetings, farming activities and social ceremonies. Furthermore, the methods of its production are simple and cheap as no elaborate equipment and expertise are required (Omueti and Morton , 1996). The *irean* and *ujumbae* production is crude, not standardized with unqualified levels of ingredients, unqualified food handlers and largely a family art.

The production of *irean* using maize involves cleaning, softening and decortications to obtain the grain grits. The grits are then milled using a plate mill and sieved to obtain the ultimate flour (Hussain, 2016). In addition, the flour is then added to boiling water in a cooking pot and stired. The mixture is done with wooden stirrer by stirring until thick-solid form is obtained. The food is ready and served in plates, food grade nylon or food take-away packs.

Production of *ujwumbae* - a guinea corn based porridge involves grinding the steeped guinea corn using grinding stone and milling machine to obtain a mashed smooth paste. The mash is gradually diluted with small quantity of water to make semi-solid form where boiling water is added in such a way to form gelatinized paste (a smooth thick form) in a local household utensil. A grinded malted rice or guinea corn or potato as sweetener

(based on individual preference) is added, mixed to and left open at room temperature overnight, and then sieved to obtain the *ujwumbae* - porridge (Onuorah *et al.,* 1987; Akuma *et al.,* 2006). The *ujumbea* is consumed either as watery on the next day or gel-like (semi-solid or thick) product that is formed immediately after steaming. Cereal-based porridges are traditionally used as baby weaning foods in many parts of the world at the first stage of weaning. The *irean* and *ujumbae* must be consumed within 18 – 36 hours of production due to poor keeping quality among other reasons (Shobha *et al.*, 2011).

The foods naturally get spoiled often during hot period due to rancidity among other factors between March and April than rainy and cool seasons– May to December as a result of lower temperature (Shobha *et al.*, 2011). The high-water content coupled with crude methods of production and packaging under improper sanitary conditions predispose the products to microbial contamination. Pathogenic and spoilage (non-pathogenic) microorganisms contaminate foods during processing (Berghofer *et al.,* 2003) and largely during serving and packaging a final stages of the products. Thus, the pathogenic organisms such as *Staphylococcus sp* that causes food poisoning*, Salmonella* causing Salmonellosis, *Shigella* - causing shigellosis or dysentery*, Escherichia causing* diarrhea, stomach pain, cramps and fever as well as fungi like *Aspergillus* are found in foods causing aspergillosis and food poisoning among others. These pathogens are known to be potential causes of food borne diseases (FBD) in humans (Hussain, 2016 and Harris *et al.*, 2012) but the beneficial ones are useful in food industry when they are harnessed in a controlled manner.

Antimicrobial Resistance (AMR) occurs when microorganisms – bacteria, fungi, viruses, and parasites – evolve to become resistant to antimicrobial substances, such as antibiotics and antifungals. This occurs naturally, through adaptation to the environment, or through selective pressure, when microorganisms come into contact with antimicrobials agents

(Food and Agricultural Organization (FAO, 2019). The process is accelerated when inappropriate or excessive use of antimicrobials occurs. As a result, medicines that were once effective treatments for particular infectious disease in people and animals become less effective or wholly ineffective, leading to a reduced ability to successful treatment of the infections. This in turn leads to more severe or prolonged illnesses, increased mortality, production losses in agriculture and reduced livelihoods and food security (FAO, 2019). Effective antimicrobials agents are critical for the treatment of diseases in people and animals, helping to ensure healthy living.

1.2 Statement of the Research Problem

The locally processed cereal foods in Kpambo community have not been fully investigated, documented and perhaps the microbial population and nutritional contents of these foods may lead to development of unhealthy conditions for the citizens. In addition, the methods of *iraen* and *ujwumbae* preparation are crude and not standardized with unqualified levels of ingredients which may lead to food contamination thus threatening the health of the consumers. The use of antibiotics by the consumers without prescription by physicians for the treatment of food related infections and others may result in multi-drug resistance by some of the pathogens, hence; prolonged healing or treatment difficulty may prevail. Antimicrobial resistance (ARM) is a current global threat to human and animal health (FAO, 2019).

1.3 Aim and Objectives of the Study

The aim of the study was to assess the microbiological and nutritional qualities of local cereal foods processed in Kpambo Community of Taraba State, Nigeria.

The objectives of this project were to:

i. enumerate and isolate microorganisms present in the locally processed cereal foods.
ii. characterize and identify the microbial isolates
iii. determine proximate nutrient composition of the locally processed cereal foods.
iv. determine antibiotic resistance profiles of the bacterial isolates.

1.4 Justification for the Study

Cereal products are widely consumed in Kpambo Community and their consumption is correlated with health benefits. Children and adolescents who eat cereal have a lower Body Mass Index (BMI) and waist circumference than those who do not eat cereal at breakfast or who skip breakfast (Ukwuru *et al.*, 2018). Similarly, those who eat cereal may benefit from positive health benefits related to satiety, body weight and the threat for chronic ailment (Ukwuru *et al.*, 2018). It is also important to determine the antimicrobial resistance of the isolates from the food samples analyzed to ascertain the antibiotics profile of the isolates, whether they are susceptible or resistance to a given antibiotics as a measure for treatment choice for food related infectious diseases.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Food Contamination

Food contamination refers to foods that are spoiled or tainted because they either contain microorganisms, such as bacteria, fungi and others substances that make food unfit for consumption (Alum *et al.*, 2016). In order word, food contamination refers to the presence of harmful chemicals, foreign particles like hair, stone and microbial toxin in food, which can cause consumer illness (FAO, 2019). The impact of chemical contaminants on consumers' health and well-being is often deceptive only after some years and prolonged exposure at low levels. Food contamination also refers to anything in food that reduces the safety or quality of food. Food may be contaminated naturally, deliberately and unintentionally. Food contamination occurs at any stage of the food chain (production, delivery and consumption level). It can result from several forms of environmental contamination including pollution in water, soil, air, as well as unsafe food storage facility, processing utensil and equipment. The microbiological contamination of cereal grains begins at the growing phase of the grains. The intensity of the contamination can increase during the growth and after harvest or production resulting in the spoilage of the final products or worse, induces foodborne illnesses in the consumers if appropriate control measures are not taken (Harris *et al.*, 2012).

2.1.1 Sources of food contamination

Sources of food contamination can be microbial or environmental, with the former being more common. Environmental contaminants that can enter the food supply chain include pesticides, heavy metals, and other chemical agents. Many opportunities exist for food to become contaminated as it is produced and distributed. To start with, bacteria are present in cereal foods and other foods such as meat and poultry products. These foods become

contaminated during preparation and may be through cross-contamination. During food processing, contamination is also possible from infected food handlers as a source. Microbes are known to be ubiquitous; therefore the products could be prone to unintentional contamination by microbes found in the environment. Oyelana and Coker (2012) listed *Pseudomonas aeruginosa, Lactobacillus plantarum, Staphylococcus aureus, Escherichia coli, Klebsiella aerogenes, Aspergillus flavus, Aspergillus niger, Penicillium oxalicum, Fusarium oxysporium, Rhizopus stolonifer, Saccharomyces cerevisiae* and *Candida albicans* as microbes found in air as source of contamination, utensils and workers are another sources of contamination. The common microbial contaminants of cereal foods are *Pseudomonas, Listeria monocytogenes, Salmonella* sp., *Shigella flexneri, Vibrio cholerae, Bacillus* sp, and *Campylobacter jejuni*. Microbial biofilms are a great threat to the food industry because most of the microbes are capable of forming biofilms in the presence of even minimal amount of moisture and nutrients in the processing facility.

The source of food contamination could also due to clinical infections resulting from the biofilm by pathogenic microbes of food industry. Apart from *Pseudomonas aeruginosa* and *Lactobacillus plantarum,* the authors Oyelana and Coker (2012) have reported that the microorganisms are also found in the water used for preparation as source of contamination. In a review, Izah *et al.* (2016) reported *Staphylococcus aureus*, *E.* coli, *Alcaligenes faecalis, Proteus, Pseudomonas, Enterobacter, Salmonella, Klebsiella, Bacillus*, *Aeromonas, Micrococcus, Citrobacter, Streptococcus, Vibrio, Shigella, Enterococcus, Flavobacterium, and Chromobacterium* species as bacteria diversity found in different potable water sources in Nigeria and this could be potentially lead to disease condition such as gastroenteritis, diarrhea and dysentery. Generally, this could have been

happening but it often goes unreported especially in rural areas (Gabriel-Ajobiewe *et al.*, 2014).

Major sources of cereal food contamination are contaminated equipment, water, air, insects, rodents, and food handlers (Ray *et al.,* 2004, Hussain, 2016). Equipment can be a source of contamination if not thoroughly clean regularly and sterilize. It can contaminate the food during production. Food production operatives (personnel) can be a source of introducing pathogens into food production areas. Producers of cereal foods and food ingredients should control the entry of staff into production areas and suitable procedures to reduce the risk of product contamination. The hand, hair, nose and mouth of food personnel carry microorganisms that can be transferred to the food during processing, preparation, packaging and serving by touching, breathing and coughing or talking.

Water may be found in many parts of a food production area and can be used in many ways. It is usual to try to reduce considerably, or eliminate, water usage in many areas such for cleaning points and as an ingredient in many processed food. In production areas, much of the microbiological control is centered on keeping these areas as dry as possible and thus preventing microbial growth. Allowing potable and non-potable water to access such areas creates a potential for microorganisms that reside in a dormant state to begin to grow. Growth can be rapid and high numbers can be reached if the area is warm. This can provide a source of contamination of the final product (Alum *et al.*, 2016).

The air can also serve as a source of contamination. Within a production environment, air may make contact with the cereal food products on many occasions and this will introduce a risk that any microorganisms present in the air may enter the product especially where

food preparation is carried out in an open area. And such factors potentially influencing the risk of air introducing pathogens into the food products (Alum *et al.*, 2016).

Pests such as insects, birds and rodents introduce a risk since they are likely carriers of pathogens that could contaminate food products. The entrance of pests of any sort into a food warehouse, dry storage and production areas introduces a risk that pathogens will also enter and contaminate finished products (Alum *et al.*, 2016). .

2.1.2 Implications of consuming contaminated foods

Food is liable to be contaminated at any stage along food chain (producer to consumer). Mostly food serves as a potential vehicle for transmission of certain diseases agents under certain circumstances especially when it gets contaminated with harmful microbes, microbial toxins and other environmental contaminants (Alum *et al.*, 2016). When food becomes contaminated, it has the potential to cause sickness and infections (Hussain, 2016). Depending on the source and level of contamination, the effects of contaminated food can cause symptoms such as cramps, nausea, diarrhea, vomiting, nerve damage, allergies, paralysis and neurological, gynecological and immunological symptoms. Implications of consumption of contaminated food can cause short-term symptoms such as nausea; vomiting and diarrhea commonly referred as food poisoning or long-term symptoms such as cancer, kidney or liver failure, brain and neural disorders (World Health Organization (WHO, 2020). Ingestion of contaminated food is a serious growing public health problem causing considerable social economic impact and contribute significantly to the global burden of disease and mortality especially the low and middle income countries (WHO, 2020).

2.2 Contamination and Spoilage of Cereal Foods

The levels of cereal contamination and spoilage with bacterial pathogens are usually very low but of concern for cereal processed product. Food spoilage is a process in which food whether cooked or raw deteriorate to the point in which it is not fit for human consumption. Microbial food spoilage occurs as a consequence of either microbial growth especially in ready to eat food or release of intracellular and extracellular enzymes in the food. Due to rapid replication nature of microorganisms as the food conditions are suitable, eventually microbial growth increases, thereby releasing metabolites (enzymes or toxin) into the food that bring about different changes such as colour, odour, texture (slime formation), and accumulation of gas and liquid in the foods (https://www.bing.com). During cooking, some microorganisms and their metabolites (toxin) are destroyed by heat while others still survive the cooking temperature of 100^0c hence causing spoilage (ttps://food.unl.edu/free-resource/food-storage). The major factors involved in the spoilage of cereal grains and products by molds are moisture levels and temperature. Cereal grains and products normally contain several genera of bacteria, moulds, and yeasts (Izah *et al.*, 2016)

The type of microorganisms found in each food depends upon the microbial ecology (relationship between microbes and their surrounds), water activity (measure of 'free' water in a food sample, as opposed to 'bound' water) and nutrient composition. The cereal food is an ideal medium for microbial growth; especially cereal foods with high moisture are good condition for microbial (spoilage) growth. Other contributing factors in cereal foods spoilage are methods used during processing, nature of packaging, time of storage and possible temperature abuse (Izah *et al*., 2016).

2.3 Nutritional Qualities of Cereal Foods

Cereals are staple foods, and are important sources of nutrients in both developed and developing countries. Proper selection of local cereal foodstuff is possible to prepare nutritious local diets that would be acceptable, readily available, affordable and nutritionally adequate. Dissemination of the findings at scientific and community levels is very desirable. Apart from moisture content and inedible substances such as cellulose, cereal grains and products contain, along with traces of minerals and vitamins, carbohydrates - mainly starches - (comprising 65 -75 percent of their total weight), as well as proteins (6 -12 percent) and fat (1-5 percent). The World Health Organization (WHO) and UNICEF have been concerned about this trend, particularly of Protein Energy Malnutrition (PEM) and micronutrient deficiencies (hidden hunger) among infants, children and pregnant women. The United Nations system Standing Committee on Nutrition (SCN) pointed out that malnutrition is directly and indirectly associated with more than 50% of all children mortality, and is the contributor to disease in developing world (SCN, 2004). Although cereal foods are generally low in fat, this subgroup contributes 7% to the average daily intake of total fat in adults and 5% in children (Gregory, 2000; Henderson *et al.* 2003). In addition, cereal foods contain niacin, riboflavin, thiamine and fibre. A common nutritional qualities of cereal foods is their high content of water, fibre, low energy and micronutrient densities (Solomon, 2000). This characteristic becomes particularly worrisome during the complementary feeding period in infants and children and are therefore known to poorly support growth and development.

Poor processing methods and hygiene have also been identified as other factors responsible for low nutrient density in local complementary foods. Processes such as milling, fermentation, and parboiling are intended to achieve specific purposes but them

however tend to affect the nutrient content of the food negatively. Okoye (1992) reported a 90% loss of free folic acid content of cereals and certain vegetables, 50% of yam thiamine and ascorbic acid and up to 20% of milk vitamin E content during boiling.

The presence of non-nutrient constituents (anti-nutritional factors) in cereal-based foods has been shown to also negatively influence the bioavailability of nutrients. The best documented being oxalic acid which forms oxalate precipitates with dietary calcium, while phytic acid forms insoluble phytates with Ca, Fe, Zn and possibly other metals. For instance the relatively poor availability of the fairly high Fe content of cereals is mainly due to their correspondingly high phytic acid levels (Okoye, 1992). The need for protein during the period of skeletal and muscle growth of early infancy is high. An intake of 2.1g of high biological-value protein per kilogram (kg) of body weight permits nitrogen retention of about 45%, as long as energy intake is adequate (Guthrie, 1989). By 5 to 6 month the protein needs drop slightly to 2g/kg. If the protein is of low biological value the amount needed increases proportionately. Protein with high biological value of at least 70 to 85%, e.g. eggs, milk, meat, with almost half of amino acids being essential amino acids, have been recommended to be used for infants (Picciano, 1987).

Protein in excess of the body's need for growth and repair of tissue must be reduced in the liver so that the carbon skeleton of the amino acids can be used as a source of energy, and the amino portion is excreted as urea. Since the infant has a limited capacity to concentrate waste metabolites, such as urea in the urine, the excretion of more waste requires a large volume of water. If the necessary water is not available, urea will accumulate, and ironically, the infant will suffer from protein edema (Guthrie, 1989).
Micronutrients are vitamins and mineral elements, which are needed in minute quantities for the normal functioning of the body. They are normal chemical components of foods

in their active forms or as precursors of the active forms. They form components of enzymes or co-factors needed for metabolic reactions in the body (Devlin, 1997).

The common practice of milling and polishing of grains, parboiling of vegetables and legumes used during the processing and preparation of foods for infants and children, have been found to reduce the levels of micronutrients (La Roche, 1990), and could cause micronutrient and other nutritional deficiency disorders in infants and children (UNICEF, 2000).

The consequences of micronutrient malnutrition in infants and children may include growth retardation, anorexia, and susceptibility to infections, behavioral changes and learning disability. The high mortality and morbidity rate among human has led to the view that the modern world should not tolerate the persistence of these deficiencies. UNICEF (2000) has put the global mortality rate of children due to "hidden hunger" at 114 per 1000 (11.4%) live births.

2.4 Microbiological Qualities of Cereal Foods

Many of the indigenous cereal-based foods produced and consumed around the world contain microorganisms that are natural (not control) and intentionally (control). Information is not always available about the microorganisms involved, which makes it very difficult to maintain the product quality (Izah *et al.,* 2016). The microflora of some traditional cereal-based foods include some of the yeasts and lactic acid bacteria. Microorganisms play both essential and deleterious roles in food products generally. In the food preparation setting, the attributes of the food products produced is largely due to the type, age composition of the microorganisms present (Izah *et al.,* 2016). Microbes found in cereal foods occur via several ways including exposure, handling, use of contaminated utensils and water during preparation packaging and serving at the final

stages of the products. Several groups of bacteria (coli-forms, lactic acid bacteria, and aerobic bacteria) and fungi (yeasts and moulds) participate in locally prepared cereal foods. The genera of these microorganisms include *Staphylococcus, Escherichia, Pseudomonas, Enterococcus, Klebsiella, Bacillus, Lactobacillus, Leuconostoc, Clostridium, Streptococcus* (bacteria) and *Aspergillus, Saccharomyces, Mucor* (fungi) (Izah *et al.,* 2016). Microorganisms are the main cause of food spoilage. The quality of raw materials, water and utensil are essential in the end- products of the processed cereal foods. In addition, cereal foods are typically held or kept at room temperature which can dramatically reduce their quality and shelf-life of the product. The packaging material could also be a source of contamination if not sterilized. Most of the cereals or cereal-based products like *irean* and *ujwumbae* contain high amount of water that could make prevention of microbial growth difficult. In order to prevent contaminations that reduce the qualities of cereal foods, different strategies such as appropriate temperature, water content, food grade packaging materials, wholesome water and awareness on food hygiene should be taken into consideration at all production stages (packaging, serving, keeping and transportation) (ttps://food.unl.edu/free-resource/food-storage).

2.5 Processing of Cereal Foods

The processing of cereal foods locally is typically carried out by small scale holders, who process it for subsistence and to a larger extent for commercial purpose (source of livelihood). The processing of *irean and ujwumbae* is carried out using rudimentary equipment and often in an un-hygienic environment especially during processing, handling and serving. The most basic processing methods for cereal foods include cleaning, milling, sieving and boiling. The principal procedure is milling—that is, the polishing and grinding of the grain so that it can be easily cooked and rendered into an attractive foodstuff (Hussain, 2016 and Makhal *et al.*, 2003). Sieving is

another process where grinded grain are passed through a fine tiny hole filter to remove chaff and obtain ultimate flour or products for cereal based products. Boiling - local cereal based foods production is usually done by cooking using fire wood and cooking pot. The contents are boiled in a pot on a three set of stone in three positions with fire under (personal observation). Other processes for processing cereal based food products are packaging, and transportation.

2.6 Importance of Cereal Foods

Cereals are staple foods available around the world throughout the year providing sources of nutrients in both developed and developing countries. These foods help in prevention of some human diseases and maintain an overall healthy lifestyle. Consumption of a cereal - based food in everyday can retain and keep the energy levels intact throughout the day. Cereals contain fibre, consuming enough fiber per meal can reduce the risk of heart blockages, stroke among other disease and also aid digestion, building body tissues and muscles (Hussain, 2016). The economic aspect of cereal foods is helpful in that there is creation of employment in the cereal food industries and factories. The rural and urban dwellers earn a living with these foods by producing varieties of food products for personal consumption and for sale. There is much interest in understanding the role of particular foods, such as cereals, in the diet and their effect or importance on health. Cereals have range of health benefits: Soluble bran in cereals is helpful for lowering blood cholesterol levels and also prevents cardiovascular, cancer, constipation, colon disorders and high blood sugar (Franko and Albertson,2010; Hussain, 2016) and reduces hunger (Franko and Albertson, 2010). Eating relatively large amounts of cereal foods significantly lower the risk of cardiovascular disease (CVD), coronary heart disease (CHD) and stroke and reducing the risk of weight gain (Hussain, 2016; FAO, 2019).

Similarly, Consumption of high-carbohydrate high-fibre improves glycaemic control for people with type 1 or 2 diabetes (Mann, 2001) and high- soluble fibre diet decrease blood glucose and insulin more than a diet of equivalent macronutrient and energy content containing moderate amounts of fibre (Chandalia *et al.*, 2000). Cereals are commonly used as ingredients in many food and other products around the world.

2.7 Prevention and Control of Cereal Food Contamination and Spoilage

Prevention of microbial contamination and spoilage is essential to decrease the rate of deterioration, wastage, food-borne diseases, prolong shelf-life of the food products (*ujwumbae* and *iraen*) and ensure safety of the foods. The contamination can be controlled by proper cleaning and sanitization of production equipment and facilities. Sterilized equipment before usage, purified water or pot-table water should be used at every step of food processing. The designing of equipment should be done carefully to avoid microbial growth as biofilm at any part of the equipment (Katepogu *et al.*, 2018). Packaging food under air control such as Modified Air Packaging (MAP) within shortest time to prevent introduction of particles and air borne droplets into the food. According to Tabit (2018), several control and prevention measures were suggested as follows: Removal of contaminated seeds before preparation, protect stored products from any conditions that favour continuing microbial growth, maintain proper hygiene (Washing of hands and tools regularly). Cooking at 100 ^{O}C, packaging and storing of processed cereal foods appropriately below 5°C prevent quick spoilage. Packaging is key to prevention of food spoilage depending a selected suitable materials for packaging a particular food, especially processed cereal products, considering factors such as moisture gain or loss, lipid oxidation that will result to off flavor and loss of freshness (Makhal *et al.*, 2003). The susceptibility of cereal products to lipid oxidation is associated with the concentration and type of fat content used and the number of unsaturated bonds in fatty acids. The

packaging must contain and preserve a certain quantity of product as efficiently as possible and also must protect its contents from external threats including spoilage, breakage, and damage from external environmental conditions, pilfering and theft (Tabit, 2018). Primary, secondary and tertiary packaging must be designed so that the product stays in perfect condition until it reaches the end user.

2.8 Packaging and Storage of Processed Cereal Foods

The packaging is a process where final or finished food products are put into a container before reaching the end user and it must contain and preserve a certain quantity of the processed cereal products as efficiently as possible and also must protect its contents from external threats including spoilage, chemical contaminants, breakage, spillage and damage from external environmental conditions. The package materials must be strong and durable enough to protect the product with a reasonable safety margin (Albertson and Thompson, 2008). Most processed cereal products are packaged with plastic and polythene - based materials – Polyethylene terephthalate (PET), leather, plates of any kind and thermo-cool container popularly known as cooler either big or small size, food flask for later consumption. These packs have unique characteristics including good strength properties, good aesthetic appearance, and environmental friendly among others such as labelling to give information about the food products including expiry date as the case may be. For immediate consumption, processed cereal foods that are in solid forms are packaged in a metal, plastic, ceramic plates while the liquid forms like *ujwumbae*, pap are packaged in a cup, Polyethylene terephthalate (PET) plastic bottles for immediate and later use. Packaging protects contents from spillage and further infestation of other contaminants – insects, rodents among others to the food. Finished processed cereal foods are usually packaged in any form of package materials before transportation and storage to avoid spillage and recontaminations of the end-products. Storage of foods basically

helps to maintain safety, keeping flavor, color, texture and nutrients in food for an extended period (shelf life of the foods) (Bender and Bender, 1999). **In Nigeria, homemade foods are usually stored in particular containers called food cooler for a short period of time** (ttps://food.unl.edu/free-resource/food-storage). **Refrigerator is another food storage facility where processed cereal foods are** kept at or below temperature 40°F (4°C) (ttps://food.unl.edu/free-resource/food-storage; (Bender and Bender, 1999). The short, but safe, time limits will keep refrigerated foods for extended periods of time rather than quick spoiling of the processed cereal food products.

2.9 Future Prospects of Cereal Foods in Nigeria

The demand for cereal products is driven by the ever changing consumer and their lifestyle. Cereals and cereal products are important human food resources and livestock feeds worldwide. Cereal production (CP) in Nigeria has great potentials that can contribute significantly to the economic growth if well harnessed. In addition, cereal products are used as ingredients in numerous products. In the recent years if not the insecurity among other conditions in Nigeria, cultivation of cereal crops has increased tremendously which in turn increases its consumption. This is actually correlated with high consumption of whole corn as popcorn, roasted or boiled corn, and cereal products such as the *irean,* and *ujwumbae* on daily basis throughout the year in Nigeria. Consumption of cereal foods bring about healthier body weights and may protect against cardiovascular disease risk (Franko and Albertson, 2010).

Cereals are used for both human and animal food and as an industrial raw material. Although milled white flour is largely used for bread production, especially in industrialized countries, the grain may be converted to food in other ways (Bezborodov *et al*., 2018). Cereals are high in starch, which may be used in pure or flour form. Starches are also obtained from such root sources as potatoes and from the pith of tropical palm

trees. Various starches are used commercially in food processing and in the manufacture of laundering preparations, paper, textiles, adhesives, explosives, and cosmetics (Dey, 2014).

The principal cereals used as components of animal feeds are wheat and such wheat by-products as the outer coverings separated in the preparation of white flour (bran and the more floury middlings), corn, barley, sorghum, rye, and oats (Kaur *et al*. 2012). These are supplemented by protein foods and green fodders. Animal foods require proper balance between the cereals (carbohydrates) and the more proteins foods, and they must also contain suitable amounts of necessary minerals, vitamins, and other nutrients. The compounded ration for a milking cow generally contains about 50–80 percent cereals, consisting of wheat by-products, flaked or ground corn, barley, sorghum, wheat, and oats (López-Sánchez *et al*., 2020). Requirements for most balanced rations for pigs and poultry are similar. Corn is especially useful in high-energy feeds either as meal or as the flaked and partly gelatinized product; barley is desirable for fattening, and oats help provide a better balanced cereal for livestock. Without cereals for use in farm animal foods, the available supply of the animal protein required in the human diet would be greatly reduced (Meleshkina *et al*., 2017).

The major cereals in Nigeria include Maize, Sorghum, Millet and Rice. Cereals are utilized in various ways in Nigeria. Maize is utilized in different ways such as ogi. Ogi is a porridge prepared from fermentation of maize in Nigeria. It is a staple food that can be served as a weaning food for infants or breakfast for adults (Ismaila *et al*., 2017). Ogi can also be prepared from millet and sorghum. Pito which is produced from maize is a traditional alcoholic drink in Nigeria (Ismaila *et al*., 2017). Popcorn (guguru), egbo, agidi, moi-moi, and tuwo are also produced from maize. Sorghum is utilized in different ways which include production of burukutu - a local alcoholic beverage in Nigeria. The

process involves malting, mashing, boiling, fermentation and maturation. It can also be produced using a combination of maize and sorghum. Kunun zaki is also produced from sorghum which can be combined with millet or maize depending on individual preference. Pito and Ndaleyi are as well made from sorghum (Ismaila *et al*., 2017).

Millet is one of the major cereals utilized in Nigeria to produce kunun zaki, fura and the process involves decortication/dehulling, washing, drying, milling, fermenting, moulding, steaming, and drying. Masa, pito,ogi are all produced from millet. Rice is one of the major cereals in Nigeria which is used to make tuwo and can be consumed with beans soup and masa. It is used to produce kunun by mixing it with millet. The tropical Africa has many cereal crops with diverse utilization most of which are not properly documented (Ismaila *et al*., 2017).

Globally, about 16% of cereal production is traded internationally in 2021, ranging from 10% for rice to 24% for wheat. The share of traded production for cereals is projected to marginally increase to 17% by 2031, largely due to increased trade shares of wheat and rice (FAO, 2022). In volume terms, net cereal surpluses and deficits show a clear regional pattern. However, these patterns differ among cereals. For example, the bulk of the exportable surplus of rice is projected to remain concentrated in Asian countries, while in Latin America and the Caribbean the export of maize is largely compensated by imports of wheat. Overall, several African and Asian countries are projected to become more dependent on cereal imports over the coming decade (Igoryanova and Meleshkina, 2017).

Export prices of cereals have been on the rise since mid-2020 and, according to FAO's Cereal Price Index, at the close of the 2021 calendar year reached a nine-year high. While price increases registered during 2020 were largely attributed to disruptions caused by the COVID-19 pandemic and a strong increase in maize imports by China, those registered

during 2021 were largely the result of sharp increases in prices of wheat and maize, which outweighed declines in international rice prices (Meleshkina *et al.*, 2021). Export quotations of wheat in 2021 rose by 31%, on average, primarily in response to tight global availabilities, especially of high-quality wheat, following reduced harvests in major producing countries, in particular Canada, the United States of America, and Russia (Vitol *et al.*, 2015).

Cereal prices are likely to remain high for the 2023-2031 marketing season but assuming average yields and geopolitical stability they could resume their long-term downward trend in real terms to 2031 (Meleshkina *et al.*, 2021). Cereal prices have been very volatile due to recent COVID-related domestic and global supply chain disruptions, Russia's war against Ukraine, animal diseases, yield variability, high fertiliser and transport costs, and the macroeconomic environment including high inflation. Those factors could of course alter the prices projected in this Outlook (Zabodalova, 2015). Additionally other elements such as trade disruptions from political instability and efforts to address domestic inflation could severely affect markets. While some countries have expressed their interest in implementing specific strategies focused on controlling domestic prices, such as stock building or export restrictions, in many cases the regulatory framework and its implementation remain unclear. Finally, cereal prices could also become more volatile given the increasing exposure of regions experiencing extreme weather events (Renzyaeva, 2021).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

Taraba is a State in North Eastern geo-political zone of Nigeria, named after the Taraba River, which traverses the southern part of the State. Taraba State's capital is Jalingo. The inhabitants are mainly the people from Fulani, Mumuye, Karimjo, and Wurkun tribes, who are predominantly resident in the northern part of the State, while the Jukun, Chamba, Tiv, Kuteb and Ichen tribes are found predominantly in the southern part of the State. The central region is mainly occupied by the Mambila people, Fulani and Jibawa. There are over 77 different tribes, and their languages in Taraba State. Taraba State is bounded in the west by Nasarawa State and Benue State, northwest by Plateau State, north by Bauchi State and Gombe State, northeast by Adamawa State, and South and Northwest Region by Cameroon (Olaitan *et al*., 2015).

The Benue, Donga, Taraba and Ibi are the main rivers in the State. These rivers rise from the Cameroonian mountains, straining almost the entire length of the state in the North and South directions to link up with the River Niger. The climate of Taraba State is marked by an annual average temperature of 33°C but high level of cold in January and an increased rainfall in August. The percentage of rainfall in Taraba State is 40.35% with 54.98% relative humidity. The State is usually very warm in March with 40.44°C, and an average wind of 8.84km/h (Olaitan *et al*., 2015).

The major occupation of the people of Taraba state is agriculture. The major crops produced in the state include maize, rice, sorghum, millet, cassava, yam, groundnuts and cotton in commercial quantity. In addition, cattle, sheep and goats are reared in large numbers, especially on the Mambilla Plateau, and along the Benue and Taraba valleys. Similarly, the people of Taraba State undertake other livestock production activities like

poultry production, rabbit breeding and pig farming in fairly large scale. Communities living on the banks of River Benue, River Taraba, River Donga and Ibi, engage in fishing all year round. Other occupational activities such as pottery, cloth-weaving, dyeing, mat-making, carving, embroidery and blacksmithing are also carried out in various parts of the State (Olalekan *et al*., 2015).

The study area is Kpambo Community in Ussa Local Government Area of Taraba State, Nigeria. Figure 3.1 is the map of Nigeria showing 36 States and FCT while Figure 3.2 is the map of Taraba State showing Ussa Local Government Area where the study area is located.

Figure 3.2. Map of Taraba State Showing Ussa Local Government Area (study area) and other Local Government Areas (LGAs) of the State

Source: Taraba State Geographical Information System (2015)

Kpambo is one of the communities of Kuteb in Ussa Local Government Area of Taraba State, Nigeria. Another name for Kpambo Community is *Ticwo*. Kpambo shared boundaries with Fikyu*, Bika, Kwambai, Rufu,* and *Lissam* Communities. The population of Kpambo Community is 30,004 while the population of Ussa is 92, 017 (National Population Commission, 2006). The people of Kpambo are predominantly farmers with few who are Government workers or business men /women. Therefore, most of the people engage in agricultural activities with the main aim of producing crops to meet their needs (livelihood). Like most parts of Northern Nigeria, Kpambo Community has wet/rainy and dry seasons. Wet season is from April to October while the dry season is from November to March. The people of Kpambo Community regularly consume locally processed cereal foods *iraen* and *ujwumbae* as their daily diet. The samples of these foods are shown in Plate 1 (*Ujumbae*) and Plate II (*iraen*).

Plate I: *Ujumbae* products packaged in plastic bottles

Plate II: *Iraen* products wrapped with nylon and placed in a plate

3.2 Collection of Samples

Freshly prepared samples of *irean (*maize-based meal) and *ujumbae* – (a guinea corn-based porridge) were collected based on the method described by Cheesbrough (2010) from food vendors in Kpambo Community for a period of four months from April to July, 2021. The samples were collected in sterile sample bottles, placed in ice box and transported to the microbiology laboratory of the National Institute for Pharmaceutical Research and Development (NIPRD), Idu-Abuja, Nigeria for analysis.

3.3 Enumeration of Microbial Counts

3.3.1 Preparation of serial dilution

One mililitre (1ml) of *ujwumbae* and one gram (1g) of *irean* samples were introduced into 9ml of sterile peptone water, homogenized and left for 15 minutes to activate microbial growth. These were subsequently serially diluted up to dilution factors of 10^6 to evenly distribute and reduced the microbial concentration (Cheeesbrough, 2010).

3.3.2 Determination of Bacteria Counts and Isolation

3.3.2.1 *Total coliform / faecal coliform counts*

This analysis was performed using the Most Probable Number method as described by Food Safety and Standard Authority of India (FSSAI, 2012). Three tubes each containing 9 ml of MacConkey broth with inverted Durham tubes were inoculated with one millilitre (1 ml) of the food homogenate. Similar operations were carried out for the first (1:100) and the second (1:1000) dilution tubes using fresh sterile syringe for each dilution. The MacConkey broth tubes were incubated at 37±1°C for 24 hours and the presumptive tests were carried out for the tubes showing gas production and colour change from purple to cream after 24 hours and those that recorded negative gas production were re-incubated for another 24 hours.

For the confirmed test, a loopful each of the tubes with positive gas production and colour change from purple to cream were transferred from each of the tubes to a separate tube of Brilliant Green Lactose Bile (BGLB) broth with similar label and were incubated at 35° C for 48 hours. The BGLB tubes with gas production in the inverted Durham tubes were confirmed positive for coliforms and were calculated from the Most Probable Number (MPN) table. Furthermore, the positive tubes were subsequently subcultured into separate plates of MacConkey agar, labelled accordingly and incubated at 35° C for 48

hours. The routine laboratory method of Cheesbrough (2010) was used to characterize different isolates.

3.3.2.2 ***Enumeration of Salmonella and Shigella species***

One milimitre each of the homogenate (1:10, 1:100, and 1:1000) were aseptically added into three Petri dishes with corresponding labelling. Fifteen millilitre of *Salmonella Shigella* Agar (SSA) was aseptically added and swirled so that the samples (*ujwumbae* and *iraen)* were evenly distributed and were allowed to gel. The plates were then incubated aerobically in an inverted position at 37 °C for 24 hours. Colonies were isolated and sub cultured on nutrient agar slant for further characterization as described by FSSAI (2012).

3.4 Characterization and Identification of Bacterial isolates

The characterization and identification of the isolated organisms was based on the colonial morphology, staining reaction (gram staining) and standard biochemical tests, as described by Cowan and Steel (2003) and Cheesbrough (2000). The unknown bacteria were further identified using Bergey's Manual of Determinative Bacteriology as described by De Ley and Frateur (2014).

3.4.1 Morphological identification of isolates

The organisms were identified based on their morphological characteristics were examined for colony colour, shape, texture and elevation with the use of naked eyes and use of hand lenses according to the methods described Cheesbrough (2000).

3.4.2 Gram staining

The Gram staining technique as described by Cheesbrough (2000) was adopted in the identification of the bacteria's Gram reaction. Bacteria smear was prepared on clean grease free slide, air dried and heat fixed. The heat-fixed smear was flooded with crystal violet for 60 seconds and was washed off rapidly with clean water. The slide was then

flooded with iodine solution and was left for another 60 seconds before rinsing with clean water. Acetone was used to rapidly decolourize the slide and was washed off immediately before counter stained with neutral red for 2 minutes. It was then washed with a clean water, blotted carefully and air dried in a draining rack. The stained smears were first examined with x40 objective lens under the light microscope to check the staining and material distribution; before using oil immersion objective lens of x100 to view the bacterial cells.

3.4.3 Biochemical characterization of the isolates

3.4.3.1 *Catalase test*

The catalase test was carried out using the standard procedure as described by FSSA1 (2012) where a clean slide was placed on clean staining rack, one drop of 3% hydrogen peroxide (H_2O_2) was placed on the centre of labelled slide using sterilized wire loop (flamed to red hot then allowed to cool). A portion of pure isolate was transferred aseptically on to each drop of hydrogen peroxide (each isolate on different labelled slide). The evolution (effervescence) of oxygen gas bubbles indicated a positive reaction while negative result produced no gas bubbles (Fawole and Oso, 2005).

3.4.3.2 *Citrate utilization test*

This test is mainly used to check the organism's ability to utilize citrate as its main carbon and energy source for growth; and ammonium salt as its sole source of nitrogen (FSSA1 (2012 and Etok *et al.,* 2004). This test was carried out using Simmons' citrate agar, which was prepared using a McCartney bottles and inoculated with the suspected organisms by streaking the agar slant and stabbing the butt using a sterile needle. The bottles were incubated at 37°C; they were examined daily for 96 hours for growth and colour change. A positive result showed a colour change in the bromothymol blue indicator, changing it from green to blue and negative when the medium retained its original green colour.

3.4.3.3 *Oxidase Test*

Using a dropper pipette, one drop of 1% aqueous solution of eramethyl -p- phyenylene diamine dihydrochloride solution was added to sterile filter paper on to which portion of the isolates was added (using sterile wire loop). A deep purple colour within 15 seconds indicated positive result, while oxidase negative would not produce this colour (Fawole and Oso, 2005).

3.4.3.4. Coagulase test

A drop of normal saline was placed on a clean-slide, and a small portion of the isolate was then emulsified onto the saline until homogenous suspension was obtained. A drop of human plasma was added to each of the suspension and stirred for 5 seconds. Coagulase positive result indicated clumping, which could not re-emulsify. Negative result showed no clumping (Fawole and Oso, 2005; Oyeleke and Manga, 2008).

3.4.3.5. Indole production test

A 24 hour-old culture of the isolates were inoculated into 1% peptone water in test tubes and incubated at 37°C for 24 hours. After incubation, 0.5 ml Kovac's reagent was added and observed for a colour change. Deep red colour indicated indole production, while orange or yellow colour indicated negative result (Cheesbrough (2000) and Hammes *et al.,* 2013).

3.4.3.6. Motility Test (stab culture technique)

An isolate was picked with sterilized straight wire and stabbed into sterile semisolid medium (motility medium) in test tubes, the stab was stopped about the centre of the medium in the test tube. This was done in duplicates for the test bacterial isolates. The test tubes were incubated at 37 °C for 24 hours, thereafter, the tubes were examined. The motile bacteria (positive growth) grew along the line of stab causing turbidity and

rendering the medium opaque, while non-motile bacteria (Negative growth) grew only along the line of stab (Cheesbrough, 2010).

3.4.3.7 *Starch hydrolysis test*

Starch hydrolysis test was carried out according to the procedures described by Cheesbrough (2000). This test is used to identify bacteria that can hydrolyse starch (amylose and amylopectin) using the enzymes amylase and oligo-1, 6-glucosidase. Duplicate plates of 1% starch agar medium was prepared and the bacterial cells aseptically inoculated on them. The plates alongside the control was incubated for 72 hours at ambient temperature. The plates were then flooded with Gram's iodine solution and observed for amylase production indicated by the holo zones formed around the test organism.

3.4.3.8 *Sugar utilization and fermentation tests*

Sugar utilization test was carried out according to the methods described by Etok *et al.* (2004). Generally, this test is used to demonstrate the ability of certain organisms to utilize and ferment some sugar medium, producing acid and gas or acid only or no fermentation at all (Etok *et al.,* 2004). The sugar medium is usually made from peptone water base containing certain volume of 10% desired sugar solution in distilled water and phenol red as an indicator. Durham's fermentation tubes were inserted in an inverted position into test tubes containing 10ml of peptone water with phenol red indicator and was aseptically plugged with sterile cotton wool. The tubes were sterilized in an autoclave at 121°C for 15 minutes. Each of the sugar solutions was filter-sterilized and the final sugar medium was made by aseptically adding 0.25mL of the sugar solutions into the sterile peptone water with indicator.

The tubes containing the sugar solutions was opened and the mouth flamed. Using a sterile wire loop, the organism was inoculated into the tubes containing each of the sugar

solutions. The tubes were flamed lightly and plugged with a sterile cotton wool and thereafter incubated at 37°C for 24 hours for the bacterial isolates.

3.5 Determination of Proximate Nutrient Composition

The determination of proximate nutrition composition of the cereal foods (*iraen* and *ujwumbae*) produced locally in Kpambo Community was carried out according to the method of Association of Official Analytical Chemists (AOAC, 2000).

3.5.1. Preparation of food homogenate

This was carried out according to the method described by FSSAI (2012). The cap of each of the sterile sample bottles was wiped with a sterile cotton wool soaked with 70% alcohol, shaken before they were aseptically opened and the neck of the bottle flamed lightly before and after collecting the samples. Samples (5mL) was aseptically transferred into forty-five millilitres of pre- sterilized Buffered Peptone Water (BPW) using a sterile pipette, mixed by shaking vigorously and carefully to harmonize the sample.

After mixing the food homogenate (1:10) by vigorous shaking, 2mL from each of the samples was aseptically transferred into 18mL of BPW contained in a 20 millilitre-capacity McCartney bottle using a sterile pipette and mixed. From the first dilution (1:100), two millilitres each of the various serially diluted samples was taken into the second dilution bottle (1:1000) containing 18mls of fresh pre-sterilized BPW and was shaken very carefully. The same procedure was repeated for the entire process up to the third dilution as described in FSSAI (2012).

3.5.2 Determination of moisture content

Moisture content was determined by weight loss of 2g of sample after heating in an oven at 105°C for 3hours. A crucible apparatus was washed, dried in an oven, cooled in a desiccator and weighed as W1. Two grams each of the samples were weighed into the cooled dried crucible and the weight recorded as W2. It was transferred into a moisture extraction oven at 105°C and heated for 3 hours. The dried samples were cooled in desiccators and were reweighed (W3). The process was repeated until constant weights were obtained according to the methods described by AOAC (2000). The difference in weight was then calculated as Percentage moisture using Equation 3.1:

$$\% \text{ moisture} = \frac{W2 - W3}{W2 - W1} \times 100 \qquad (3.1)$$

Where:

w1 = weight of empty crucible

W2 = weight of crucible + samples before heating.

W3 = weight of crucible +sample after heating.

3.5.3 Determination of crude protein

The Micro Kjeldahl method described by Association of Official Analytical Chemists (AOAC, 2000) was used in the determination of the crude protein content of the cereal food samples collected from Kpambo Community. It involved three main stages: Digestion, Distillation and Titration.

3.5.3.1 *Digestion*

Two grams of each of the samples was mixed with 10 millilitres of concentrated H_2SO_4 in eleven heating tubes. One gram of copper sulphate and a speck of selenium catalyst was added into each of the tubes and the mixture heated in a Kjeldahl heating block inside a fume cupboard for 10 hours till the mixture became colourless. The digests were

transferred with several washings into a 50-millilitre volumetric flask and was made up to the mark with distilled water.

3.4.3.3 ***Distillation***

The Micro Kjeldahl distillation apparatus was steamed through for 15 minutes, after which 10 millilitre portions of the digest was poured into the apparatus via the small funnel aperture and was mixed with equal volume (10 mL) of 40% NaOH solution. The mixtures were distilled/steamed through for 7 minutes to collect enough ammonium sulphate. The distillates were collected into 5mL volume of 2% boric acid solution mixed with 3 drops of methyl red indicator contained in a 100 mL conical flask positioned under the condenser tip. The procedure was repeated twice for each of the samples in triplicates.

3.5.3.3 ***Titration***

Each of the triplicate distillates for the samples collected in the receiving flasks was titrated using 0.1N hydrochloric acid and the average value taken. The Nitrogen content was calculated and multiplied by 6.25 to obtain the crude protein content. This is given as percentage Nitrogen using Equation 3.2:

$$\% \text{ Nitrogen} = \frac{V_S - V_B \times N\text{-acid} \times 0.01401 \times 100}{W} \qquad (3.2)$$

Crude Protein = % Nitrogen x F

Where:

V_S =Volume (ml) of the acid required to titrate the sample

V_B = Volume (ml) of the acid required to titrate the blank N-acid= Normality of the acid (0. IN)

W = weight of the sample used (g)

F = Conversion Factor (6.25 for cereal food samples and others)

3.5.4 Determination of fat

Fat content was determined by extraction method using a glass soxhlet based on **extraction with either ethyl ether and petroleum ether and gravimetric determination of the extraction residue following NIPRD standard operating procedures (SOP)** described by Werner Schmidt method of Fat Acid Hydrolysis (Kirk and Sawyer, 2017; AOAC, 2000). The principle involves digesting the proteins with concentrated Hydrochloric acid. Liberated fat is extracted with alcohol, ethy**l ether and petroleum ether. Ethers are evaporated and residue left behind is weighed to calculate the fat content.** Five grams (5g) of the sample designated as 'W' was heated with 10ml of concentrated Hydrochloric acid in a boiling water bath to dissolve the fat, the heating continued until the mixture became brown. It was allowed to cool and was transferred into a separating funnel. Ten millilitres of ethanol was added followed by 25ml of diethyl ether. The mixture was shaken vigorously for one minute and was allowed to stand until upper liquid was practically clear in order to separate the fat content of the sample.

Thereafter, a clean dried conical flask was weighed as W1, and the ether layer was transferred into the flask. The extraction was repeated twice with 25 ml of diethyl ether and the solvent evaporated in a water bath and subsequently dried at 100°C in an oven, cooled and weighed as W2.The Percentage (%) Fat content was calculated using Equation 3.3:

$$\% \text{ fat} = \frac{100\,(W2 - W1)}{W} \quad (3.3)$$

Where:

W1 = Weight in g of contents in the extraction flask

W2 = Weight in g of contents in the flask bowl after removal of fat

W = Weight in g of sample taken for the test.

3.5.5 Determination of carbohydrate

The Soluble Carbohydrate content often referred to as Nitrogen Free Extract (NFE) was determined using the methods described by Association of Official Analytical Chemists (AOAC, 2000). The carbohydrate content was calculated as weight by difference between 100 and the summation of other proximate parameters (% Ash, % crude Protein, % Crude Fat, % Crude Fibre and % Moisture content) as described by (Kirk and Sawyer, 2017).

$$\% \text{ Carbohydrate content} = 100 - (M + P + A + F + F_2)$$

Where:

M = Moisture content

P = protein content

A = Ash content

F = Crude fat content

F_2 = Crude fibre content

3.5.6 Determination of ash

Platinum crucibles were washed, dried in an oven, cooled in a desiccator and weighed as W_1, two grams of each of the samples was weighed into the cooled dried crucibles and the weights recorded as W_2 and was evaporated to dryness in a water bath before being charred over a hot plate placed in a fume cupboard until no sooths was given off. They were transferred using a pair of tongs into a muffle furnace set at 55^0C till fully ash (colour change to grey) and free of carbon. The samples were removed from the furnace, cooled in a desiccator to room temperature and reweighed immediately (W_3). The procedure was repeated twice to account for the triplicate readings as described by AOAC (2000). Ash was determined using Equation 3.4:

$$\% \text{ Ash} = \frac{W_3 - W_1}{W_2 - W_1} \times 100 \qquad (3.4)$$

Where:

W_1 = weight of empty crucible

W_2 = weight of crucible + sample

W_3 = weight of crucible + ash.

3.5.7 Determination of crude fibre

Two grams (2g) of the sample and lg asbestos was added into 200ml of 1.25% of H_2S04 and boiled for 30 minutes. The solution and content were poured into Buchner funnel equipped with muslin cloth and secured with elastic band. This was allowed to filter and residue was put into 200ml boiled NaOH and boiling continued for 30 minutes, then transferred to the Buchner funnel and filtered. It was then washed twice with alcohol, the material obtained was washed thrice with petroleum ether.

The residue obtained was collected in a clean dry crucible and dried in the moisture extraction oven to a constant weight. The dried crucible was removed, cooled and weighed. Then, difference of weight (i.e. loss in ignition) was recorded as crucible fibre (F_2) and expressed in percentage using Equation 3.5:

Percentage crude fibre:

$$F_2 = \frac{W_1 - W_2}{W_3} \times 100 \quad (3.5)$$

Where:

W_1 = weight of sample before incineration

W_2 = weight of sample after incineration

W_3 = weight of original sample

F_2 = Crude fibre

3.6 Determination of Antibiotic Resistance Profile of the Bacterial Isolates

Loopful of each pure isolate was prepared in a 3mL of normal saline within 15 minutes to activate growth prior to inoculation onto nutrient agar plate. Antimicrobial

susceptibility test using a Kirby-Bauer agar disc diffusion method (CLSI, 2006) was employed. A suspension of each was prepared in peptone water to standardize and activate the inoculums and to match 0.5McFarland turbidity Standard. Standardized inoculum of each isolate was inoculated onto a surface of plain Mueller-Hinton agar plate. An impregnated antimicrobial diffusion disc (Himedia) of 8 different concentrations were placed onto the plate within 15 minutes to avoid over population of the isolates and were incubated at 37^oC for 24 hours. The zone of inhibition was measured in millimetre (mm).

3.7 Data Analysis

The data obtained (occurrences of isolates, proximate nutrition content and antibiotic susceptibility and resistance pattern of the isolates) were subjected to percentage, statistical Analysis of one way Variance (ANOVA) at 95% level of confidence ($P \leq 0.05$) employing Duncan Multiple Range Test. The significant values were determined using the IBM Statistical Package for Social Science (SPSS) version 23 at the Degree of Freedom, $P < 0.05$. Statistical differences between means was compared using paired Duncan HSD. Differences in means were considered statistically significant at $p < 0.05$.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1. Results

4.1.1 Microbial counts and identity in cereal foods

Table 4.1 shows the total microbial load of *ujwumbae* samples. The results revealed that the samples were highly contaminated with bacteria. Total aerobic bacterial counts (TABC) ranged from 1.0 x10^2 cfu/mL to 6.4 x10^4cfu/mL while total coliform counts (TCC) ranged from 1.4 x10^2 cfu/mL to7.8 x10^2 cfu/mL.

Table 4.1: Total microbial counts of *ujwumbae* samples analysed (cfu/mL)

Sample Code	TABC (cfu/mL) ×10^3	TAFC(cfu/mL)	TCC (cfu/mL) ×10^2
A1	6.3 x10^3	ND	3.0 x 10^2
A2	4.5 x 10^3	ND	2.0 x 10^2
A3	1.3 x 10^2	ND	2.4 x 10^3
A4	ND	ND	2.5 x 10^2
A5	1.0 x 10^2	ND	1.4 x 10^2
A6	2.0 x 10^2	ND	7.0 x 10^2
A7	6 .4 x 10^4	ND	7.8 x 10^2
A8	10.7 x10^2	ND	ND
A9	3.1 x 10^2	ND	ND
A10	2.3 x 10^3	ND	ND

Note: TABC = Total aerobic bacterial count, TCC = total coliform count, A1 – A10 = *ujwumbae* samples and ND= Not Detected.

Table 4.2 shows the total microbial load of *irean* samples analyzed. It was observed that the TABC ranged from1.3 x10^6cfu/g to 8.3 x 10^8cfu/g while the TCC ranged from1.0 x10^3 cfu/g to 8.0 x10^5 cfu/g. No fungi were detected in the samples.

Table 4.2: Total microbial load of *irean* samples analysed (cfu/q)

Sample Code	TABC (cfu/g)	TAFC (cfu/g)	TCC (cfu/g)
B1	ND	ND	1.0 x 10^3
B2	1.3 x 10^6	ND	ND
B3	5.8 x 10^6	ND	6.2 x 10^4
B4	1.4 x 10^6	ND	2.9 x 10^4
B5	6.0 x 10^8	ND	4.0 x 10^5
B6	9.0 x 10^5	ND	2.0 x 10^5
B7	7.0 x 10^8	ND	1.0 x 10^4
B8	2.0 x 10^8	ND	ND
B9	6.0 x 10^8	ND	8.0 x 10^5
B10	8.3 x 10^8	ND	ND

Note: TABC = total aerobic bacterial count, TAFC = total aerobic fungi count, TCC = total coliform count, B1 – B10 = *iraen* samples and ND= Not Detected.

Table 4.3 shows the characteristics and identity of microorganisms found in the processed cereal foods analyzed. The results revealed that the identified microorganisms in the local cereal food samples analyzed were *E. coli* - a rod shape, indole positive organism; *S. aureus – a* golden yellow small cluster cocci shape, Gram positive, catalase positive as well as coagulase positive bacteria; *P. aeruginosa* - a large pinkish Gram negative rod shape on cetrimide agar and oxidase positive bacteria; *K. oxytoca* – a Gram negative pinkish rod shape and indole positive bacteria and *Klebsiella pneumoniae* – a Gram negative pinkish rod shape and indole negative.

Table 4.3: Characterization and identification of bacteria found in *ujwumbae* and *irean* samples

Bacteria	Morphology	Biochemical Characteristics						
		Cit	Oxi	Coa	Cat	Ind	Mot	H_2S
Escherichia coli	Gram -ve rod, Pinkish, LF, Moist, Raise colonies on MCA.	-ve	-ve	-ve	-ve	+ve	-ve	-ve
Staphylococcus aureus	Gram + ve cocci in cluster, golden yellowish small colonies on MSA.	-ve	-ve	+ve	+ve	-ve	-ve	-ve
Pseudomonas aeruginosa	Gram -ve rod, pinkish, LF, raise, smooth and large colony on TSA, glucose fermentation -ve and cetrimide agar +ve.	-ve	+ve	-ve	-ve	-ve	-ve	-ve
Klebsiella oxytoca	Gram –ve rod, Pinkinsh, LF, Moist, Raise colonies on MCA and MR-VP +ve.	–ve	–ve	–ve	–ve	+ve	–ve	–ve
Klebsiella pneumonia	Gram –ve rod, Pinkinsh, LF, Moist, Raise colonies on MCA and MR-VP –ve.	–ve	-ve	-ve	-ve	-ve	-ve	–ve

Note: -ve = negative, +ve = positive, LF= lactose fermentation, MCA = Maconkey agar, Cit= citrate test, Oxi= oxidation test, Coa = coagulase test, Cat= catalase test, Ind= indole test, Mot= motility test and H_2S = hydrogen sulfide test.

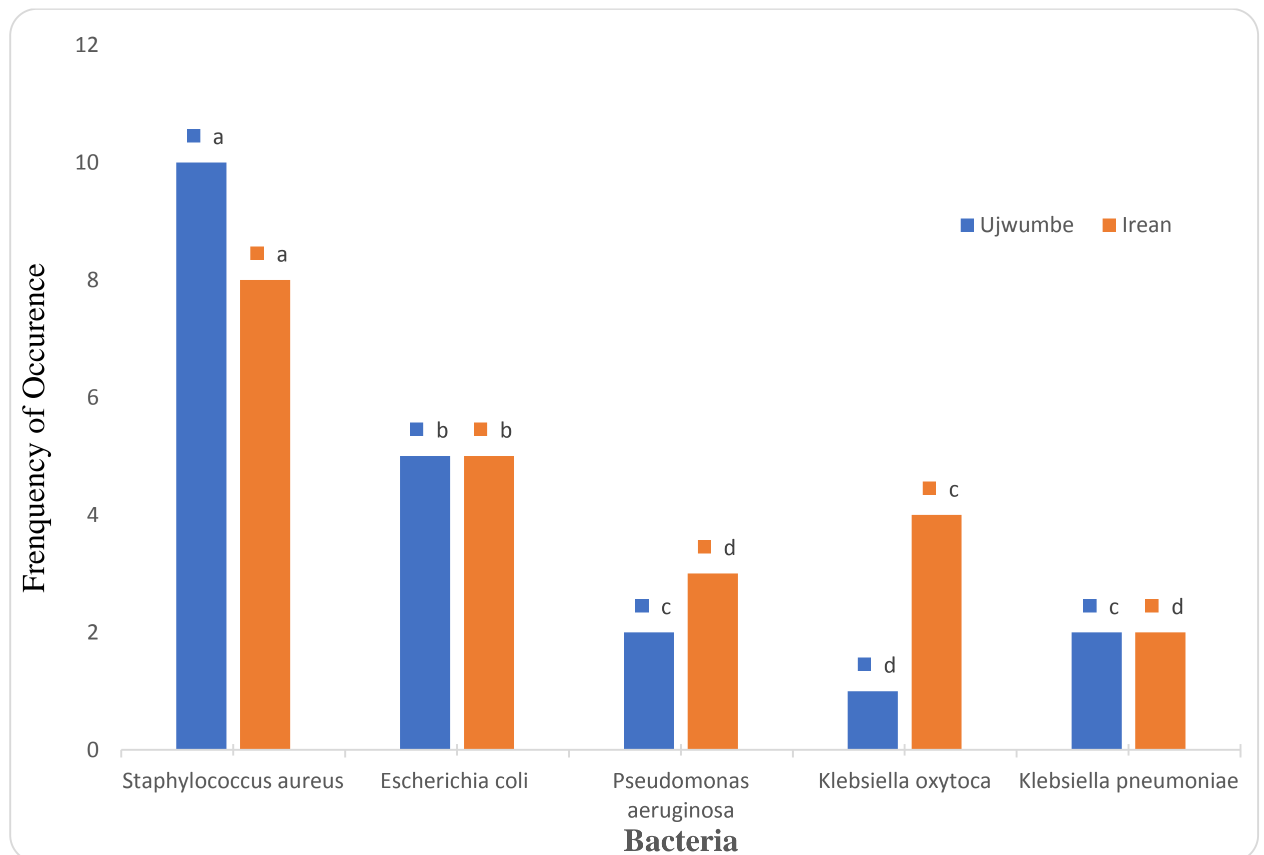
Table 4.4 indicates the presence and absence of bacterial isolates in local processed cereal foods obtained from Kpambo Community. The results revealed that *S. aureus* were present in all the cereal food samples that were analyzed except in two samples - B7 and B9; *E. coli* were present in nine samples; *P. aeroginosa* and *K. oxytoca* were isolated in five samples respectively and *K. pneumoniae* were the least (only present in four samples) in the food samples analyzed.

Table 4.4: Distribution of bacterial in *ujwumbae* and *irean* samples

	Samples																			
Bacterial	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**	**A9**	**A10**	**B1**	**B2**	**B3**	**B4**	**B5**	**B6**	**B7**	**B8**	**B9**	**B10**
Staphylococcus aureus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	_	+	_	+
Escherichia coli	+	+	+	_	_	+	_	_	+	_	+	_	_	+	_	+	+	_	_	+
Pseudomonas aeruginosa	_	_	_	+	_	_	+	_	_	_	_	_	+	_	_	+	_	_	+	_
Klebsiella oxytoca	_	+	_	_	_	_	_	_	_	_	_	+	_	_	_	+	+	_	_	+
Klebsiella pneumoniae	_	_	_	+	_	_	_	_	+	_	_	+	_	_	+	_	_	_	_	_

Note: + = present (detected); - = absent (not detected); A1 – A10 = *ujwumbae* samples B1 – B10 = *irean* samples.

Figure 4.1 shows the percentage frequency of occurrence of bacteria in *ujumbae* and *iraen* samples obtained from Kpambo Community and the analyses revealed as follows: *S. aureus (*50% in *ujwumbae*, 36.36% in *iraen*); *E. coli* (25% in *ujwumbae*, 22.73% in *iraen)*; *P aeroginosa (*10% in *ujwumbae*, 13.64% in *iraen)*; *Klebsiella oxytoca (*5% in *ujwumbae*, 18.18% in *iraen)* and *Klebsiella pneumoniae* (10% in *ujwumbae,* 9.1% in *iraen)* samples analyzed.



Values are means of duplicate determinations. Means with dissimilar letter (s) differ significantly according to the Duncan Multiple Range Test.
*Significant at $p \leq 0.05$; **Significant at $p \leq 0.01$

Figure 4.1: Frequency of occurrence of bacteria isolated in local processed cereal foods (*iraen* and *ujwumbae)*

4.1.2 Proximate nutritional composition of the locally processed cereal foods obtained from Kpambo

Table 4.5 shows the percentage proximate nutritional contents of *ujwumbae* samples analyzed. The results revealed that the moisture content of *ujwumbae* samples ranged from 93.90% to 96.99% which differed significantly according to Duncan Multiple Range Test at $p \leq 0.05$; ash ranged from 0.04% to 0.34% which differed significantly at $p \leq 0.05$; while protein ranged from 3.0% to 4.0% and were significantly different at $p \leq 0.05$. It also observed that fat ranged from 0.40% to 0.54% and carbohydrate ranged from 0.07% to 2.24%. These values were significantly different according to Duncan Multiple Range Test at $p \leq 0.05$. No fibre was detected in the *ujwumbae* samples analyzed (Table 4.5).

Table 4.5: Proximate nutritional contents of *ujwumbae* samples analyzed

Sample	Moisture (%)	Ash (%)	Fiber	Protein (%)	Fat (%)	CH (%)
A1	$96.99^{a}\pm0.00$	$0.34^{e}\pm0.00$	ND	$3.03^{a}\pm0.00$	$0.45^{b}\pm0.00$	ND
A2	$96.02^{a}\pm0.20$	$0.19^{c}\pm0.00$	ND	$3.23^{c}\pm0.00$	$0.49^{c}\pm0.00$	$0.07^{a}\pm0.00$
A3	$94.30^{b}\pm0.00$	$0.14^{c}\pm0.00$	ND	$3.00^{a}\pm0.00$	$0.45^{b}\pm0.00$	$2.11^{b}\pm0.00$
A4	$94.52^{b}\pm0.07$	$0.10^{b}\pm0.00$	ND	$3.15^{b}\pm0.00$	$0.50^{c}\pm0.00$	$1.73^{c}\pm0.00$
A5	$95.15^{c}\pm0.05$	$0.24^{d}\pm0.00$	ND	3.23 ± 0.00	$0.40^{a}\pm0.00$	$0.98^{b}\pm0.00$
A6	$93.9^{b}\pm0.02$	$0.15^{c}\pm0.00$	ND	$3.15^{b}\pm0.00$	$0.54^{d}\pm0.00$	$2.19^{b}\pm0.00$
A7	$93.90^{b}\pm0.21$	$0.24^{d}\pm0.00$	ND	$3.43^{c}\pm0.00$	$0.40^{a}\pm0.00$	$2.03^{d}\pm0.00$
A8	$93.90^{b}\pm0.00$	$0.24^{d}\pm0.00$	ND	$3.75^{d}\pm0.00$	$0.40^{a}\pm0.00$	$1.71^{c}\pm0.00$
A9	$93.76^{b}\pm0.01$	$0.04^{a}\pm0.00$	ND	$3.7^{d}\pm0.00$	$0.50^{c}\pm0.00$	$2.24^{b}\pm0.00$
A10	$96.44^{a}\pm0.02$	$0.10^{b}\pm0.00$	ND	$4.0^{d}\pm0.00$	$0.49^{c}\pm0.00$	ND

Values are means of duplicate determinations. Means with dissimilar letter (s) differ significantly according to the Duncan Multiple Range Test.
*Significant at $p \leq 0.05$; **Significant at $p \leq 0.01$

Note: A1 – A10 = *ujwumbae* samples, CH= carbohydrate. ND =Not detected

Table 4.6 shows proximate nutritional contents of *irean* samples analyzed**.** The results revealed the nutritional contents were in this order: moisture ranged from 70.90% to 83.35% which differed significantly according to Duncan Multiple Range Test at $\boldsymbol{p \leq 0.05}$; ash contents were between 0.09% and 1.25% while the protein ranged from 4.75% to 6.30%. These values were significantly different according to Duncan Multiple Range Test at $\boldsymbol{p \leq 0.05}$: It was also observed that the fat content ranged from 1.55% to 2.36% while the carbohydrate ranged from 9.47% to 20.84% and were significantly different according to Duncan Multiple Range Test at $\boldsymbol{p \leq 0.05}$. No fibre was detected in the samples (Table 4.6).

Table 4.6: Proximate nutritional contents of *irean* samples analyzed

Sample Code	Moisture (%)	Ash (%)	Fiber (%)	Protein (%)	Fat (%)	CH (%)
B1	70.90^{a}±0.00	0.10^{a}±0.00	ND	5.83^{b}±0.00	2.36^{d}±0.00	20.81^{d}±0.00
B2	82.89^{e}±0.00	0.10^{a}±0.00	ND	5.03^{a}±0.00	1.88^{b}±0.00	10.10^{a}±0.00
B3	77.87^{d}±0.00	0.10^{a}±0.00	ND	5.86^{b}±0.00	1.70^{b}±0.00	14.47^{b}±0.00
B4	77.34^{d}±0.00	1.25^{b}±0.00	ND	6.30^{c}±0.00	2.01^{c}±0.00	13.10^{b}±0.00
B5	74.95^{c}±0.00	0.46^{c}±0.00	ND	6.30^{c}±0.00	1.55^{a}±0.00	16.74^{c}±0.00
B6	83.35^{e}±0.00	0.64^{d}±0.00	ND	4.75^{a}±0.00	1.79^{b}±0.00	9.47^{a}±0.00
B7	71.71^{a}±0.00	0.28^{c}±0.00	ND	5.11±0.00	2.06^{c}±0.00	20.84^{d}±0.00
B8	72.44^{b}±0.00	0.19^{b}±0.00	ND	4.87^{a}±0.00	2.25^{d}±0.00	20.25^{d}±0.00
B9	73.36^{b}±0.00	0.10^{a}±0.00	ND	4.95^{a}±0.00	2.05^{c}±0.00	19.54±0.00
B10	75.92^{c}±0.00	0.09^{a}±0.00	ND	4.91^{a}±0.00	1.96^{c}±0.00	17.12^{c}±0.00

Values are means of duplicate determinations. Means with dissimilar letter (s) differ significantly according to the Duncan Multiple Range Test.
*Significant at $p \leq 0.05$; **Significant at $p \leq 0.01$,
B1 – B10 = *Irean* samples, CH= Carbohydrate, ND= Not Detected.

4.1.3 Anti-biotic resistance profile of bacterial isolated in locally produced cereal food (*Ujwumbae* and *Irean*) samples from Kpambo

Table 4.7 indicates zones of inhibition in millimeter (mm) of growth of bacteria caused by antibiotics used. The results showed that the zones of inhibition ranged from 10mm to 45mm at different concentrations of the test antibiotics used (Table 4.7).

Table 4.7: Susceptibility Profile of Gram-positive bacteria isolated from the cereal foods (*ujwumbae* and *iraen*) Samples

Isolates	Zone of inhibition in millimeter (mm)							
	AMC (10mcg)	**CN (10 mcg)**	**CIP (10mcg)**	**CD (10mcg)**	**COX (10mcg)**	**COT (10mcg)**	**E (15mcg)**	**TE (30mcg)**
S.aUJ01	18	21	30	27	18	15	17	15
S.aUJ02	20	25	21	21	30	25	30	25
S.aUJ03	20	20	30	26	27	21	19	19
S.aUJ04	20	20	25	30	15	21	19	20
S.aUJ05	15	23	33	27	15	34	21	25
S.aIR01	24	26	25	25	25	25	26	26
S.aUJ06	15	25	35	26	15	31	21	32
S.aUJ07	27	25	26	25	25	27	27	25
S.aUJ08	13	25	25	33	33	33	15	30
S.aIR02	20	20	25	28	30	26	21	24
S.aIR03	18	19	25	20	25	27	26	20

Note: AMC = Amoxicillin/clavunalic acid, CIP= Ciprofloxacin, E= Erythromycin, TE = Tetracycline, COX= Cloxacilin, COT= Cotrimazole, CN= Cefazolin, CD= Clindamycin, S.aUJ01 – S.aUJ08 = *Staphylococcus aureus* from *ujwumbae* samples S.aIR01 – S.aIR04 = *Staphylococcus aureus* from *iraen* samples

Table 4.8 showed the resistance and susceptibility pattern of the Gram positive bacterial isolates from the cereal foods. The results revealed that out of four *S.areus* isolated from *iraen* samples, one was resistant to amoxicillin (10mcg), ciprofloxacin (10mcg), cloxacilin (10mcg), erythromycin (15mcg), tetracycline (30mcg), cotrimazole (10mcg), cefazolin(10mcg) and clindamycin (10mcg) while three isolates were susceptible to all the antibiotics used. In the case of eight numbers of *S. aureus* isolated from *ujwumbae* samples, 8(100%) were susceptible to Ciprofloxacin (10mcg), Cloxacilin (10mcg), Cefazolin (10mcg) and Clindamycin (10mcg).

Table 4.8: Resistance and susceptible pattern of the Gram positive bacterial isolates from the cereal foods

Antibiotics	**Bacteria**			
	SaIR (n=4)		**SaUJ (n=8)**	
Antibiotics/SP	R (%)	S (%)	R (%)	S (%)
AMC	1(25.0)	3(75.0)	4(50.0)	4(50.0)
CN	1(25.0)	3(75.0)	0(0.0)	8(100.0)
CIP	0(0.0)	4(100.0)	0(0.0)	8(100.0)
CD	1(25.0)	3(75.0)	0(0.0)	8(100.0)
COX	1(25.0)	3(75.0)	4(50.0)	4(50.0)
COT	1(25.0)	3(75.0)	1(12.5)	4(87.5)
E	2(50.0)	2(50.0)	6(75.0)	2(25.0)
TE	1(25.0)	3(75.0)	2(25.0)	6(75.0)
P-value	0.000	0.000	0.000	0.000

AMC = Amoxicillin (10mcg), CIP= Ciprofloxacin (10mcg), E= Erythromycin (15mcg), TE = Tetracycline (30mcg), COX= Cloxacilin (10mcg), COT= Cotrimazole (25mcg), CN= Cefazolin (10mcg), CD= Clindamycin (20mcg), S.aUJ = *Staphylococcus aureus* from *ujwumbae* samples S.aIR = *Staphylococcus aureus* from *iraen* samples, R = resistant, S = susceptible and n= number of isolates.

Table 4.9 shows the zone of inhibition (in millimeter) of Gram negative bacteria caused by antibiotics tested. The results revealed that the zone of inhibition of the various bacteria -*E. coli, P. aeruginosa, K. oxytoca* and *K. pneumoniae* ranged between 10mm and 40mm (Table 4.9). *E.coli, P. aeruginosa, K. oxytoca* and *K.pneumoniae isolated* from the samples analyzed were susceptible to ceftriaxone (30mcg), gentamycin (10mcg), cotrimoxazole (25mcg), levofloxacin (5mcg), neticilin (30mcg), and tetracycline

(30mcg*).* *E. coli* isolated from *ujwumbae* samples were resistant to amoxicillin (30mcg), and ofloxacin (5mcg), *K. oxytoca* from *iraen* sample also showed resistant to ofloxacin (5mcg) and ceftriaxone (30mcg) Table 4.9).

Table 4.9: Susceptibility Profile of Gram- Negative bacteria isolated from the cereal foods (*ujwumbae* and *iraen*) Samples

Isolates	**Diametre Zone of inhibition (mm)**							
	CTR - 30mcg	**GEN - 10(mcg)**	**COT - 25mcg**	**LE-5mcg**	**NET-30mcg**	**TE30-mcg**	**AMC-30mcg**	**OF-5mcg**
EcoliUJ01	20	26	26	26	26	25	10	10
EcoliIR01	20	26	20	15	18	20	19	15
P.aIR01	15	15	10	18	15	14	20	14
P.aUJ01	30	30	30	30	30	30	30	30
EcoliUJ02	30	11	13	33	22	11	17	31
K.oIR01	25	13	13	17	14	15	15	25
K.oUJ01	15	10	22	29	25	10	10	13
K.pIR01	33	15	15	16	15	25	20	23
K.pIR02	11	22	17	29	13	26	15	27
P.aIR02	20	26	20	20	20	14	19	20
P.aUJ02	10	15	40	40	20	18	20	10

Note: CRT= Ceftriaxone, GEN=Gentamicin, COT= Cotrimoxazole, LE= Levofloxacin, NET= Neticilin
TE= Tetracycline, AMC = Amoxicillin, OF= Ofloxacin, EcoliUJ01=*E. coli* isolated from *ujwumbae* samples, EcoliIR01= *E. coli* from *iraen*, P.aUJ01=*Pseudomonas aeruginosa* from ujwumbae, K.oUJ01= *Klebsiella oxytoca* from *ujwumbae* and K.pIR01= *Klebsiella pneumoniae from iraen samples*.

4.2 Discussion of Results

4.2.1 Microbial counts and identity in cereal foods

Microbes found in food products generally occur through several means including exposure to contaminated air, food handlers, utensils and water. However, locally processed *ujwumbae* and *iraen* samples undergo cooking and cooking temperature of 100°C kills mesophiles that grow within the range of 20 - 40^{0} C and all bacteria that are pathogenic to humans and warm blooded animals are mesophiles while spore-forming bacteria (*Clostridium* species - *C. perfringens, C. botulinum*); *Bacillus* species (*B. cereus, B. subtilis*) are heat-resistant – thermophiles that survive cooking temperatures (Alum *et al.,* 2016). Several groups of bacteria (coliforms, lactic acid bacteria, aerobic bacteria) participate in the food spoilage and deterioration) which reduces the quality and safety of the food.

The aerobic bacteria identified in the freshly prepared *ujwumbae* and *iraen* samples obtained from Kpambo Community were *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella oxytoca* and *K. pneumoniae* while the coliform bacteria identified were *Escherichia coli*. The presence of these bacteria is in agreement with the report of Nwokoro and Chukwu (2014) who reported that *Staphylococcus aureus* is an aerobic ubiquitous, and as a normal flora of the skin and nasal cavity of human as main source of contamination (Montville and Matthews, 2008; FDA, 2012), it might have been un-hygienically introduced to the food after cooking because *S. aureus* does not survive cooking temperature of 100° C (Montville and Matthews, 2008; FDA, 2012). *S. aureus* is uniquely resistant to adverse conditions such as low a_w, high salt content and osmotic stress (Montville and Matthews, 2008). In response to low a_w, several compounds accumulate in the bacterial cell, which lower the intracellular a_w to match the external a_w (Montville and Matthews, 2008). *S. aureus*' introduction to the food might also be direct

contact of personnel harboring the bacteria to the food during washing of utensils prior to and during serving of the food through talking and touching part of the food (known as cross-contamination). Packaging processes of these foods is done in an open environment without control system that might have contributed to the presence of the bacteria in the food samples (Alum *et al.,* 2016).

This also explains the presence of *Pseudomonas aeruginosa* and *Klebsiella* which require oxygen and are normally found in soil, water and can be isolated from the skin, throat and stool of healthy persons (Izah *et al.*, 2016). Within a production environment, soil may make contact with the food products on many occasions and this will introduce *Pseudomonas aeruginosa* and *Klebsiella* present in the soil to enter the food products. Factors potentially influencing the risk of air introducing these pathogens into the food products were due to poor sanitation facilities such as hand washing, and waste water drainage or points of the production environment coupled with the production that is practiced in open place (outside - an exposed areas where incoming air with soil and dust particles blown directly to the food). Open place or outdoor food preparation facilitate access of pests (insects, birds, rodents, etc.) that could further increase the risk of food products contamination with *Pseudomonas aeruginosa* and *Klebsiella* (Izah *et al.*, 2016). Allowing water to access production areas creates a potential for soil and water microorganisms like *Pseudomonas aeruginosa* and *Klebsiella* that reside in a dormant state to begin to grow once in the food samples. Growth can be rapid and high numbers can be reached as the cereal foods are good media for their proliferation. This is possible because often, no proper drainage system for waste water from hand washing and utensils points within the production area. Similarly, *Escherichia coli* isolated from the cereal foods can be attributed to the fact that most of the water used for food preparation and washing of hands or utensils in Kapambo Community are non-potable and might have

been contaminated with feaces that contain *E. coli* (Alum *et al.,* 2016). This might have happened during packaging where food packer is deep in water before scrubbing the food to avoid food gumming to the packer and serving because *E. coli* is a facultative anaerobe (capable of growing either in presence or absence of oxygen) and a mesophile which can be killed by cooking temperature (Alum *et al*., 2016). *Escherichia coli* are food indicator organisms known to cause diarrhea in human (Alum *et al.,* 2016).

The highest percentage occurrence of *Staphylococcus aureus* in the cereal food samples analyzed depicts its high contamination level as it relates to hygiene practices by food operators since *S. aureus* is a normal flora of human. This could be attributed to poor hygiene of the preparatory environment, poor hygiene practices of the food handlers during serving, packaging and handling of the foods (Katepogu *et al.*, 2018). Similarly, the low percentage occurrence of *Klebsiella* and *Pseudomonas* isolates could be linked to the fact that they are soil and water related organisms and could have been highly reduced or destroyed by cooking temperature since their entrance is mostly from the environment and contaminated water to the cereals before processing and perhaps immediate collection of the freshly prepared food samples had limit the exposure time of the food to air and soil that could have contaminated the food more.

4.2.2 Proximate nutritional composition of the locally processed cereal foods obtained from Kpambo

The reported nutritional components of the cereal food *iraen* and *ujwumbae* samples analyzed depends on the analytical method employed. However, the high moisture content is important factor for the proliferation of food-spoilage microorganisms. Scientific investigation has reported that high moisture content in foods encourages microbial growth; hence, food spoilage (Temple *et al.*, 1996) while low moisture content in food samples increases the keeping quality of the food (Alozie *et al.*, 2009). The cereals

foods - *iraen* and *ujwumbae* analyzed are not devoid of protein as many people presume. The *iraen* samples had protein contents of 4.75% - 6.30% while *ujwumbae* had 3.0% - 4.0%. However, the cereal foods fell below good source of protein as reported by Pearson (1976) that any plant foods that provide more than 12 % of its calorific value from protein are considered good source of protein. Thus, *ujwumbae* and *irean* contain reasonable amounts of protein - a body building nutrient. This is similar to the work carried out by Izah *et al* (2016) who reported protein content of cereal based foods to be in the range of 8.58-12.39 %. Another study found that the protein content of maize and guinea corn based foods in Nigeria were in the range of 10.67- 11.27 % (Ijabadeniyi and Adebolu, 2005). Iken *et al.* (2002) reported mean protein content of 10.8 %, 11.1 % and 10.5 % for cereal-based food (*ujwumbae, brukutu* and *ogi* respectively). Proteins have a major role in the growth and maintenance of the human body and are along with carbohydrates and fat (lipids), the energy giving nutrients in the diet which is an essential building block of human cells (Wu *et al.,* 2014). They are frequently called the building blocks of life because they pose a wide range of other functions in the body (cells, bones, muscles, blood, hormones, and enzymes), such as enzymatic activity, provide structural support, regulate the passage of biochemical substances across the cellular membranes (Wu *et al.*, 2014).

The *irean* samples had higher fat content compared to *ujwumbae.* This could be due to hulling, grinding and sieving processes that had removed (polished) some portion of the grains during flour making (used for production of *irean* and *ujwumbae).* The finding is slightly different from that of Mustafa *et al*; 2003 and Ikram *et al*; 2010 who reported higher crude fat content in maize and guinea corn based foods as 3.21% - 7.71 % and 3.58 - 4.47 % respectively. Fat contributes to the energy value of foods, thereby providing

essential fatty acids for optimum neurological, immunological and functional developments in children (Izah *et al.*, 2016).

Carbohydrates are the major food components of the cereal foods. The *ujwumbae* had less carbohydrate content compared to the *irean*. This is in agreement with the acidic values (pH) of *ujwumbae* (see appendix One). It could be that the Lactic acid bacteria (LAB) such as *lactobacillus* in the food might have converted the carbohydrate content of the *ujwumbae* to lactic acid as microbial population increased in the food medium. Ijabadeniyi and Adebolu (2005) reported higher values (65.63-70.23 %) of carbohydrate for maize varieties grown in Nigeria while carbohydrate in guinea corn was reported to be between 68.34 and 69.65 % (Mustafa *et al.,* 2012). Less content of carbohydrate could result to no satiety by consumers, no weight gain and perhaps inhibit multiplication of other microorganisms that do not tolerate lactic acid.

In the case of crude fibre in this study, no fibre was detected in the cereal foods (*ujwumbae* and *irean*). This could be connected to the hulling, polishing as well as fine sieve used for sieving or removal of the outer portion of the grain before production. However, studies have shown that crude fibre of cereal foods ranged from 0.80 to 2.32% (Ikram *et al.,* 2010). The fibre content of food samples can have some biological beneficial effects such as laxative effect on the gastrointestinal tract (GIT), increased faecal bulk and reduction in plasma cholesterol level in human (Okoye, 1992).

Ash is an index of mineral contents. The ash content of the cereal foods was found in the range of 0.04 % - 0.34 % (Table 4.7) for *ujwumbae* samples and 0.09 % - 1.25 % (Table 4.8 for *iraen* samples). This implies that the food samples contained a greater proportion of non-endosperm material because ash values indicate the level to which non-endosperm components are present. However, this could be due to the fact that the outer part of the

cereal grains used for *ujwumbae* and *iraen* production contain traces of minerals and could have been removed during hulling and sieving (Ikram *et al.,* 2010).

4.2.3 Anti-biotic resistance profile of bacterial isolated in locally produced cereal food (*Ujwumbae* and *Irean*) samples from Kpambo

Antibiotics are substances either in liquid or powder form used to inhibit or eliminate the growth of bacterial agents. The Gram positive and Gram-negative bacteria isolated from the freshly prepared *ujwumbae* and *irean* samples produced in Kpambo, Taraba State responded to the various concentrations of antibiotics employed. Gram-positive isolates particularly *Staphylococcus aureus* were susceptible to all antibiotics tested. The susceptibility of this isolate implies that the antibiotics were effective against the bacteria. It could be due to the fact that the number of colonies picked were minimum dose to be inhibited by the drug; it could also be that the bacteria were not hospital associated from the food handlers where use of antibiotic is abused, and hence resistance could have developed. This could also support the fact that 24 hours culture, 25mL /100mm plate, Mueller-Hinton agar, inoculation and placement of antibiotics was done within 15 minutes as recommended for antibiotics susceptibility test/screening (Clinical Laboratory Science Institute, CLSI, 2006).

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The microbiological and nutritional quality of the cereal based foods - *ujwumbae* and *iraen* collected from Kpambo community revealed the bacterial contaminants. The bacteria contaminants identified were *E. coli, S. aureus, P. aeroginosa* and *K. pneumoniae,* probably introduced during sieving and packaging of the products, as *ujwumbae* and *iraen* production is largely a family art where people involved in production, sieving and packaging do not take necessary precautions.

The cereal based - *ujwumbae* and *iraen* had considerable amounts of crude protein (3 – 6.30%), ash (0.04 – 1.25%), fat (0.40 – 2.36%) and carbohydrate (0.07 – 20.84%) but no fibre was detected in the samples.

The bacterial isolates were highly sensitive to antibiotics tested, especially Ciprofloxacin, Cefaxolin and clindamycin.

5.2. Recommendations

Based on the findings of this study the following recommendations are made:

i. Bacterial contaminants of *ujwumbae* and *iraen* should be checked by adopting good hygiene practices during preparation and handling of the products.
ii. *Ujwumbae* and *iraen* are rich in protein, fat, carbohydrate and therefore, are ideal for human consumption if not contaminated by pathogenic bacteria.
iii. *Ujwumbae* and *iraen* cannot be recommended for cholesterol reduction in the body because they contain little or no fibre.
iv. The bacterial contaminants were highly inhibited by Ciprofloxacin, cefazolin and Clindamycin. Thus, these could be drugs of choice for the treatment of food borne diseases caused by the bacterial contaminants of the products.

5.3 Contribution to Knowledge

The microbiological and nutritional quality of the cereal based foods - *ujwumbae* and *iraen* collected from Kpambo community revealed the presence of *E. coli, S. aureus, P. aeroginosa, K. oxytoca* and *K. pneumoniae*, probable introduced during sieving and packaging of the products, as the method of *ujwumbae* and *iraen* production is crude and largely a family art where the food operators do not take necessary precautions.

The cereal based foods - *ujwumbae* and *iraen* had considerable amount of crude protein (3 – 6.30), ash (0.04 – 1.25), fat (0.40 – 2.36) and carbohydrate (0.07 – 20.81), are ideal for human consumption if not contaminated by pathogenic microorganisms but the food products cannot be good for cholesterol reduction in the human body because *ujwumbae* and *iraen* contain little or no fibre.

The bacterial isolates were highly sensitive to the antibiotics tested, especially Ciprofloxacin, Cefazolin, and Clindamycin. Thus, these could be drug of choice for the treatment of food borne diseases caused by the bacterial contaminants of the food products.

REFERENCES

Abduraham, H. & Kolawale H. (2006). Evaluation of oyster mushroom (*Pleurotus ostreatus*) production using water hyacinth (*Eichhornia crassipes*) biomass supplemented with agricultural wastes. *International Journal of Food Science,* 92, (1), 23-43.

Akuma, U. I., Makinde, F.M. & Akinoso, R. (2006). Comparison between the nutritional qualities of flour obtained from raw, roasted and fermented sesame (*Sesamum indicum L.*) seed grown in Nigeria. *Acta Science Polish Technological Aliment*, 13(3), 309-319.

Albertson, A. M. & Thompson D. (2008). Consumption of breakfast cereal is associated with positive health outcomes, Evidence from the National Heart, Lung, and Blood Institute Growth and Health Study. *Nutrition Research* (28), 744-752.

Alozie, Y. E., Iyam, M. A., Lawal, O., Udofia, U & Ani, I. F. (2009). Utilization of Bambara ground flour blends in bread production. *Journal of Food Technology*, 7(4), 111-114.

Alum, E.A., Urom, S. M. O. C. & Ben, C. M. A. (2016). Microbiological contamination of food, the mechanisms, impacts and prevention. *International Journal of Science Technology Research*, 5(3), 65-78.

AOAC (2000). Official Methods of Analysis of AOAC International, AOAC international, Gaithersburg, Md, 18th edition, 2005.

Ayo, Y. U. & Okaka M. (1998). Effects of roasting temperature and time on healthy nutriceuticals of antioxidants and total phenolic content in Iranian sesame seeds (*Sesamum indicum L.*). *Iranian Journal of Environmental Health Science*, 7, 97-102.

Bender, D.A & Bender, A. E. (1999). Benders' Dictionary of Nutrition and Food Technology, 7th edn. Wood head Publishing, Abington. Bingham SA, Day NE, Luben R et al. (2003) Dietary fibre in food and protection against colorectal cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC): an observational study. Lancet 361: 1496–501.

Berghofer, B., Num, G. & Amoves, J. (2003). Quality evaluation of composite bread produced from wheat, maize and orange fleshed-sweet potato flours. *American Journal of Food Science and Technology*, 2(4), 109-104.

Bezborodov, A.M., Zagustina, N.A. & Popov, O.V. (2018). Enzymatic processes in biotechnology. Moscow, Nauka. P 335.

Cerna-Cortes, D.K., Chavan, J.K., Adsule, R.N. & Kadam, S.S. (2015). World oil- seeds, chemistry, technology, and utilization. Van Nostrand Reinhold, NewYork, USA, pp 554.

Chandalia, G, Rekha, S.S, & Pushpa, R.K. (2000). Cereal foods, A review of Indian Experience. *Food and Nutrition Bulletin*, 23(2), 67-69.

Cheesbrough, M. (2010), District Laboratory Practice in Tropical Countries. Cambridge University Press, Cambridge, UK. Pp. 243 – 244.

Cheesbrough, M. (2000) District Laboratory Practice in Tropical Countries, Part 2. Camridge University Press, 434 pp.

Clinical Laboratory Science Institute, CLSI (2006). Improving young children's diets during the complementary feeding period," in UNICEF Programming Guidance, p. 76, New York.

Cowan, G. L. & Steel, R.S. (2003). Advance in cereal science and technology, 1st ed. American Association of cereal chemist Inc, U.S.A. pp 350, 352.

De Ley, U. I. & Frateur, P. (2014). The application of protein concentrates from locally available legumes in development of cereal foods. *Journal of Nutritional Science Ernahrungswisse*, 25(2), 220-227.

Devlin, R.M. (1997). Proximate composition and anti-nutrient properties of breakfast cereals made from blends of local rice, soybeans and defatted coconut flour. *Journal of Nutritional Food Science*, 5 (11), 006.

Dey, S.S. (2014). Optimization of the production of shrimp waste protein hydrolysate using microbial proteases adopting response surface methodology. *Journal of Food Science and Technology*, 51(1), 16-24. DOI, 10.1007/s13197–011–0455–4

Etok, A. R. A., Ajayi, F.F., Alaka, O.O. & Akande, O.O. (2004). Effects of Soybean diet preparations on acid secretion and experimental ulceration in the rat. *African Journal of Medical Science*, 7(2), 23-28.

FAO (2022). OECD-FAO Agricultural Outlook" OECD Agriculture statistics (database), http,//dx.doi.org/10.1787/agr-outl-data-en

FAO. (2019). Averting risks to the food chain – A compendium of proven emergency prevention methods and tools. Second edition. Rome. Pp 104. Licence, CC BY-NC-SA 3.0 IGO www.fao.org/food-chain-crisis.

Fawole, H. & Oso, V. (2005). Nutritional evaluation of a locally formulated cereal food, JUTH-PAP. *West Africa Biological Science*, 3(4), 85-93

FDA (2012). Revised guidelines for the assessment of microbiological quality of processed foods. Department of Health, Food and Drug Administration, Republic of Philippines, pp 5-6.

Food Safety and Standard Authority of India (FSSAI) (2012). Microbiological analysis principles and specific applications. Khubab State University, India.

Franko D. L. & Albertson, A. M., (2010). Cereal consumption and indicators of cardiovascular risk in adolescent girls. *Public Health Nutrition,* 19, 1-7.

Gabriel-Ajobiewe , H., Takashi, S. & Seib, P.A. (2014). Paste and gel properties of prime corn and wheat starches with and without native lipids. *Cereal Chemistry*, 65(6), 474-483.

Gregory, D.A. (2000). Chemical Analysis of Foods (7th edition). Churchill Livingstone, UK, pp 487-573.

Guthrie, A.R. (1989). Flavonoid and condensed tannins from the leaves of *Vaccinum raticulatia* and *Vaccinum caloyimum*. Pacific Science, 48(4), 458-463.

Hammes, Y. U. Fashakin, J.B & Ogunsola, F. (2013). The utilization of local foods in the formulation of cereal foods. *Journal of Tropical Pediatrician*, 28, 93-96.

Harris, N. M., Okaka, J.C and Potter, N.N. (2012). Functional and storage properties of cow- pea powder and wheat flour blend in bread making. *Journal of Food Science,* 42, 822-833.

Henderson, H. J., Oladele, K.A. & Oberlas D. (2003). Influence of processing techniques on the nutrients and antinutrients of Tigernut (*Cyperus esculentus L.*). *World Journal of Dairy and Food Science* 4(2), 88-93.

Hu, F.B. (2003) Plant-based foods and prevention of cardiovascular disease: an overview. *American Journal of Clinical Nutrition* 78: 544S–51S.

Hussain, H. (2016). Laboratory manual for examination of water, waste water and soil. VCH Verlagsgellscaft, Weinheim (Federal Republic of Germany) and VCH Publishers, New York, USA, pp 85-124.

Idem, H. I. & Showemimu GI. (2004). Production and quality assessment of gluten-free and nutrient dense biscuits from acha, pigeon pea and sweet potato blends. *International Journal of Food and Nutritional Sciences*, 2(2), 129-139. Https,//doi. org/10.15436/2377-0619.15.030

Igoryanova, N.A. & Meleshkina, E.P. (2017). Possibilities of using secondary grain processing products to produce ingredients with food fibers. *Bakery products*, 10, 41–44. (In Russian)

Ijabadeniyi, A. O & Adebolu, T. T. (2005). The effect of processing methods on the nutritional properties of Ogi produced from three maize varieties. *Journal of Food, Agriculture & Environment*, 3, 108- 109.

Iken, J. E., Amusa, N. A & Obatolu, V. O. (2002). Nutrient composition & weight evaluation of some newly developed maize varieties in Nigeria. *Journal of Food Technology in Africa, (7), 27-29.*

Ikram, U., Muhammad, A & Arita, F. (2010). Chemical & nutritional properties of some maize (*Zea mays L.*) varieties grown in NWFP, Pakistan. *Pakistan Journal of Nutrition*, 9(11), 1113-1117.

Ismaila, M. B., Obizoba, I.C & Anyika, J.U. (2017). Nutritive value of Baobab milk (*Adanso- nia digitala L.*) Hungary rice, *Acha (Digitaria exillis)* flours. *Plant Food Human Nutrition,* 46(2), 156-165.

Izah, S. C., Kigigha, L. T & Okowa I. P. (2016). Microbial quality assessment of fermented maize Ogi (a cereal product) & options for overcoming constraints in production. *Biotechnological Research*, 2(2), 81-93.

Katepogu, I.E, Tahir, F & Abgo, E.B. (2018). Assessment of Novel Medium Synthesized from Garri, Banana Peels & Bean Chaff for Cultivation of *Sacchromyces cerevisiae & C&ida albicans*. *Nigerian Journal of Microbiology*, 3(1), 56-59.

Kaur, G., Sharma, S., Nagi, H.P.S & Dar, B.N. (2012). Functional properties of pasta enriched with variable cereal br&s. *Journal of Food Science & Technology*, 49(4), 467–474. DOI, 10.1007/s13197–011–0294–3.

Kirk, U & Sawyer, O. (2017). Nutritional value of "ogi", A Nigerian infant food. *Journal of Tropical Medical Hygiene,* 70 (2), 279-81.

La Roche, O. B (1990). Production of 'Ogi'from Germinated Sorghum supplemented with Soya beans. *African Journal of Biotechnology*, (9) 7114 – 7121.

López-Sánchez, J., Ponce-Alquicira, E., Pedroza-Islas, R., de la Peña-Díaz, A. & Soriano-Santos, J. (2020). Effects of heat & pH treatments & in vitro digestion on the biological activity of protein hydrolysates of Am- aranthus hypochondriacus L. grain. *Journal of Food Science Technology*, 53(12), 4298–4307. DOI, 10.1007/s13197–016–2428–0.

Makhal, E. A. Banma, J. Nuamah, K. A. M, Oldham J. H & Nnaji,C. O. (2003), Formulation of Cereal Food from Fermented Maize, Rice, Soybean & Fishmeal. *Pakistan Journal of Nutrition,* 8 (11), 1747-1752

Mann, V. (2001). Nutritional Enhancement of Ghanian Cereal Meal. *African Journal of Food Agriculture, Nutrition & Development.* 14(5) 99 -101.

Meleshkina, E.P., Pankratov, G.N., Vitol, I.S., K&rokov, R.H & Tulyakov, D.G. (2017). Innovative trends in the development of advanced triticale grain processing technology. *Foods & Raw Materials,* 5(2), 70–82. DOI, 10.21179/2308–4057–2017–2–70–82.

Meleshkina, E.P.,Vitol, I.S. & K&rokov, R. K. (2021). Products of triticale grains as an object for enzymatic modification Storage & processing of farm products, 9, 14–18. (In Russian)

Montville, M. J & Matthews, J.E. (2008). Malnutrition in developing countries, the challenges to the responsibilities of the experts. *Nutritional Social Group of India*, (37), 29-38.

Mustafa, T.D., Fesphamde, S & John, C. (2012). Studies on a wheat based amylase rich food .*Food Nutrition* Bulletin. 1(2), 55 - 59.

National Population Commission (2006). Archived of Nigerian Population. Retrieved from http://www.npc.gov.ng. Accessed July, 3rd, 2022.

Nkama, B & Gbenyi, N.S. (2001). Some properties of corn grains & their flours I, Physicochemical, functional & chapati-making properties of flours. *Food Chemistry* 101(3), 938-946.

Nwokoro, Y. U & Chukwu, J. F. (2014). Morphology & culture in the prehistoric new world, Westview press, Boulder pp 101-112

Okoye, Z.S.C. (1992). Biochemical aspects of nutrition. Prentice-Hall of India, New Delhi, 147- 195.

Olalekan A, Bashir, A & Abdul-Mumini, K. (2015). Physico-chemical characteristics of borehole water quality in Gassol Taraba State, Nigeria. *African Journal of Environmental Science & Technology*, 1(2), 23-24

Omueti, O. & Morton I.O. (1996). Development, Acceptability Nutritional & Physical Characteristics of Protein improved Nigeria Abari (Maize Meal) prepared from Soya & Maize flours. *International Journal of Food & Nutrition, 47(5): 369 – 375.*

Onuorah, G., Ingbian, E.K & Akpapunam, M.A (1987). Appraisal of traditional technologies in the processing & utilization of mumu; a cereal based local food product. *African Journal of Food Agricultural Nutritional Development* 5(2), 1-18.

Oyelana, H. J & Coker, D.K. (2012). Functional properties of the great Northern bean (*Phareolus vulgaris L.*) Proteins - Emulsion foaming, viscosity & gelation properties. *Journal of Food Science*, 46, 71-81. https, //doi. org/10.1111/j.1365-2621.1981.tb14533.x

Oyeleke, Y. U & Manga, E.F. (2008). Weaving practices & their outcome, a critical Look with special reference to Ghana. *Bulletin of Noguchi Memorial Institute for Medical Research*, 4(2), 3-24.

Pearson D. (1976). The chemical analysis of foods, 17th ed., Churchill Living Stone, London. 3-4.

Picciano J.H. (1987). Principles & procedures of statistics. *A biometrical approach*, 2nd edition, McGraw Hill Book Company, New York, USA, pp 44.

Ray, H., Onwuka, G.I & Himma, R. U. (2004). Food analysis & instrumentation, theory & Practice, Napthali Press, Lagos, Nigeria, pp 133-137.

Renzyaeva, T. V., Tuboltseva, A.S., Ponkratova, E. K., Lugovaya, A.V & Kazantseva, A. V. (2021). Functional & technological properties of powdered raw materials & food additives for confectionary Food Processing. *Techniques & Technology*, 4(35), 43–49.

SCN (2004). Laboratory method for food & dairy Microbiology. *Academic Press*, 18(3), 226-227.

Shobha, Y., Okeke, J.N.C., Ozumba, A.U., Olatunji, O.O & Odunfa, S.A. (2011). Effect of drying methods on some qualities of Nigerian *Pleurotus pulmonarius* mushroom powder. *Nigerian Food Journal*, (21) 137-143.

Solomon, D. (2000). Phytates. Intoxicants occurring naturally in foods. National Academy of Sciences, pp 363-371.

Tabit, E. (2018). Improvement of cereal practices for the future. *Bulletin of Noguchi Memorial Institute for Medical Research*, 4(1), 82-92.

Taraba State Geographical Information System (2015). Taraba State archived. Retrieved from http://Nigeriagallery/tarabastate/gis.gov.ng. accessed 23rd September, 2021

Temple, V. J., Badamosi, E. J., Ladeji, O & Solomon, M. (1996). Proximate chemical composition of three locally formulated complementary foods. *West African Journal of Biological Sciences*, (5) 134-143.

Ukwuru, M. U., Muritala, A & Iheofor, A. O. (2018). Cereal Utilization in Nigeria. *Research Journal of Food & Nutrition, 2*(3), 1-12.

UNICEF (2000). Protein Advisory Group of the United Nations -PAG guideline no.8, Protein- rich mixtures for use as cereal foods. New York.

Vitol, I.S., Karpilenko, G.P & Kazakov, E.D. (2015). Modification triticale flour using a proteolytic Enzyme Preparations. Storage & processing of farm products,(9), 17–22. (in Russian)

WHO (2020). Vitamin & Mineral Requirements in Human Nutrition, National Academy Press, Washington, DC, 2020.

Wu, Y., Gordon, D.T & Field, M.L. (2014). Release of phosphorus from phytate by natural lactic acid fermentation. *Journal of Food Science*, 48(1), 933-54.

Zabodalova, L.A. (2015). Scientific foundations of functional products. St. Petersburg, *University ITMO*. —86 p. (in Russian).

APPENDIX I

pH of freshly prepared *ujwumbae* and *iraen* sample

The pH of freshly prepared *ujwumbae* and *irean* samples collected from Kpambo, Taraba State showed that guinea corn-based porridge (*ujwumbae*) had pH ranges from 3.13 to 3.73 and *irean* 3.41 to 6.38. Basically, different microbes tolerate pH differently, to some it encourages their growth while in others it antagonizes and leads to their death. However, the highly acidic condition of *ujwumbae* samples could be connected to the activities of Lactic acid bacteria. Although, Lactic acid bacterial (LAB) were not food indicator organisms and were not intended to be isolated while that of maize-based meal (*irean*) is almost neutral except one that is acidic. This is, however, as a result of continual increase in population of lactic acid bacteria. LAB usually turn medium acidic and, thus, antagonizes the proliferation of other groups of microorganisms.

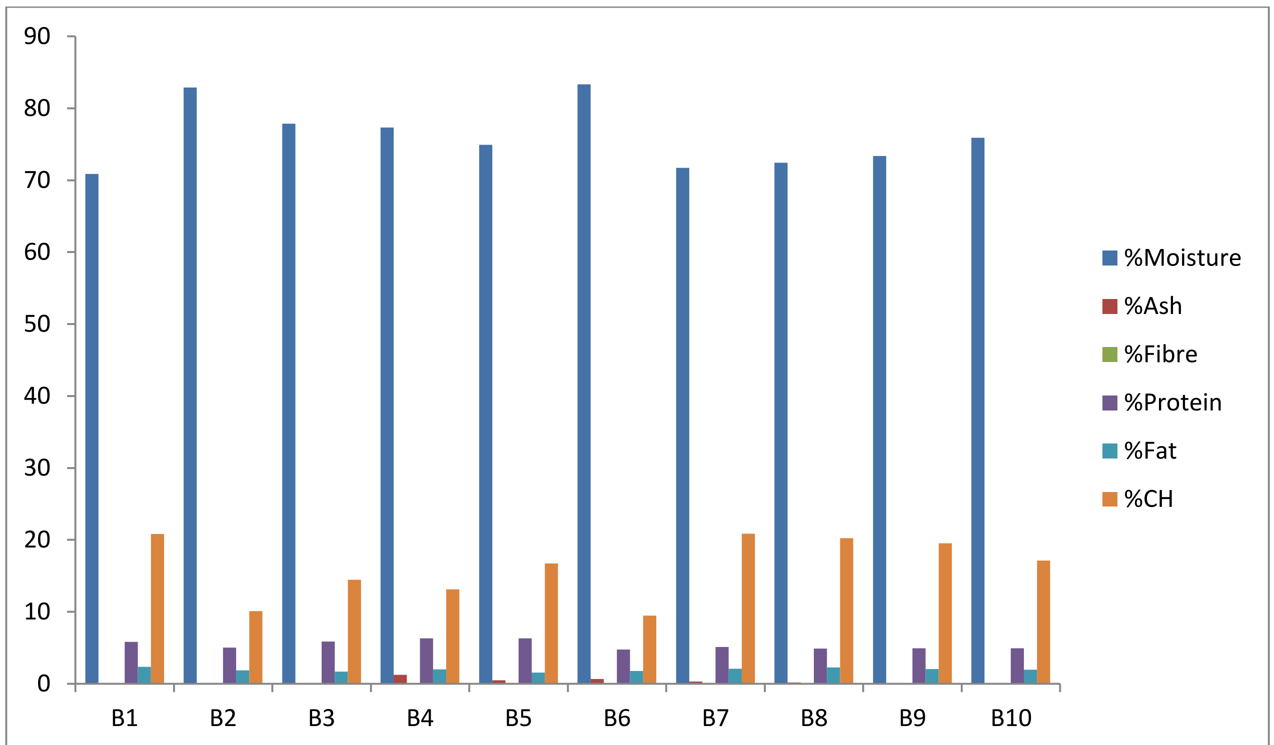
pH of *Ujwumbae* and *Iraen* Samples

Sample	pH	Sample	pH
A1	3.66	B1	6.38
A2	3.73	B2	6.33
A3	3.69	B3	6.21
A4	3.72	B4	5.06
A5	3.13	B5	4.21
A6	3.16	B6	3.41
A7	3.46	B7	6.15
A8	3.47	B8	5.61
A9	3.41	B9	5.67
A10	3.41	B10	5.78

Note: A1 – A10 = *ujwumbae* samples code, B1 – B10 = *iraen* samples code

APPENDIX II

Proximate nutritional contents of *irean* samples analyzed



Appendix Three

Distribution of bacteria isolated from *ujumbae* and *iraen* samples locally processed from Kpambo Community

The percentage frequency of bacterial occurrence in *ujumbae* and *iraen* samples obtained from Kpambo Community and the analyses revealed as follows: *S. aureus* - 50% in *ujwumbae* and 36.36% in *iraen* samples; *E. coli* - 25% in *ujwumbae* and 22.73% in *iraen* samples; *P aeroginosa* – 10% in *ujwumbae* and 13.64% in *iraen*; *Klebsiella oxytoca* – 5% in *ujwumbae* and 18.18% in *iraen*; and *Klebsiella pneumoniae* – 10% in *ujwumbae* and 9.1% in *iraen* samples analyzed.

Table 4.4 Distribution of bacteria isolated from *ujumbae* and *iraen* samples locally processed from Kpambo Community

Isolates	***Ujwumbae***	***Iraen***
Staphylococcus aureus	10 (50%)	8 (36.36%)
Escherichia coli	5 (25%)	5 (22.73%)
Pseudomonas aeruginosa	2 (10%)	3 (13.64%)
Klebsiella oxytoca	1 (5%)	4 (18.18%)
Klebsiella pneumoniae	2 (10%)	2 (9.09%)
Total	20 (100%)	22 (100%)