KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

COLLEGE OF SCIENCE

FACULTY OF BIOSCIENCES

DEPARTMENT OF FOOD SCIENCE

WATER ACTIVITY PROFILE AND MICROBIAL SAFETY OF HOT PEPPER SAUCE "SHITO"

BY

GLORIA ASAFO-ADJAYE

NOVEMBER, 2018

WATER ACTIVITY PROFILE AND MICROBIAL SAFETY OF HOT PEPPER SAUCE "SHITO"

By

GLORIA ASAFO-ADJAYE

(BSc Catering and Hospitality Education)

A thesis submitted to the Department of Geography, Kwame Nkrumah University of Science and Technology, Kumasi in partial fulfilment of the requirements for the award of

MASTER OF SCIENCE IN FOOD QUALITY MANAGEMENT

NOVEMBER, 2018

DECLARATION/ CERTIFICATION

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the award of any other degree or diploma at Kwame Nkrumah University of Science and Technology, Kumasi or any other educational institution, except where due acknowledgment is made in the thesis.

GLORIA ASAFO-ADJAYE **(PG1048517)** ....
Student Name and ID Signature Date

Certified by Supervisors:

Dr.(Mrs). Gloria Ankar-Brewoo ....
Signature Date

Certified by

Dr Faustina Dufie Wireko-Manu. ....

Head of Department: Signature Date

ABSTRACT

Hot pepper sauce "shito" is one of the commonly used products in most Ghanaian homes, schools, restaurants etc, which sometimes serves as condiments. This study therefore seeks to determine the water activity profile and microbial safety of hot pepper sauce „shito". "Shito" samples were purchased in the Kumasi Metropolis from Food and Drugs Authority registered and non-registered manufacturers. Water Activity and microbial contamination were determined using standard methods. Water Activity (a_w) for the results for FDA registered products were between 0.51 and 0.88 while those for product not registered with the FDA were between 0.52 and 0.95. FDA Registered shito samples recorded a significant difference lower total aerobic count (23%) as compared to unregistered samples (46%). The morphological characterization on the agar plates indicated the presence of *Escherichia coli*. and some suspected *Enterococci faecalis* spp. However, no *Salmonella*spp. was detected in the samples. A direct correlation was found between total aerobic count and coliform assay for samples, which failed the TAC assay. The presence of *E.coli* indicated the poor personal hygiene of the some of the shito manufacturers. "Shito" produced by FDA registered caterers are safer and might have longer shelf life due to their lower a_w and microbial load as compared to non-FDA registered caterers.

ACKNOWLEDGEMENT

To God be the Glory great things He has done. For without him this study would not be a success. With unqualified appreciation, I say thank you Dr. Gloria Ankar-Brewoo for the continuous support and encouragement throughout the entire study. May God never cease to bless you, lastly, I say thank you to my husband Michael Asafo-Adjaye and my children for your lovely, care and support. Thank you all once again.

TABLE OF CONTENT

DECLARATION/ CERTIFICATION ..... **II**
ABSTRACT ..... **III**
ACKNOWLEDGEMENT ..... **IV**
TABLE OF CONTENT ..... **V**
LIST OF TABLES ..... **VIII**
LIST OF FIGURES ..... **IX**
LIST OF PLATE ..... **X**

CHAPTER ONE 1
INTRODUCTION 1
1.1 BACKGROUND OF THE STUDY 1
1.2 STATEMENT OF THE PROBLEM 4
1.3 OBJECTIVE 4
1.4 SIGNIFICANCE OF THE STUDY 4
1.5 LIMITATIONS OF THE STUDY 5
CHAPTER TWO 5
LITERATURE REVIEW 5
2.1 THE CONCEPT OF WATER ACTIVITY PROFILE 6
2.2 THE IMPACT OF WATER ACTIVITY ON THE SHELF LIFE OR STABILITY OF FOODS 7
2.3 THE ROLE OF WATER ACTIVITY IN ENSURING QUALITY AND FOOD SAFETY 8
2.4 FACTORS THAT INFLUENCE MICROBIAL SAFETY IN FOOD PRODUCTS9 8
2.4.1 MICRO OR INTERNAL FACTORS 9
2.4.2 MACRO OR EXTERNAL FACTORS 10
2.5 MICROORGANISMS IN FOOD 12
2.5.1 MICROBIAL QUALITY OF CONDIMENTS 14
2.6 THE CONCEPT OF HOT PEPPER SAUCE „SHITO“ 14
2.7 MICROBIAL LOAD OF FOODS SOLD IN SHOPS AND ON STREETS IN GHANA 14
2.8 SOURCES OF FOOD CONTAMINATION 15
CHAPTER THREE 15
METHODOLOGY 15

3.1 MATERIALS 16
3.2 SOURCE OF SAMPLE 16
3.1 WATER ACTIVITY DETERMINATION 16
3.2 SAMPLE PREPARATION 16
3.3.1 PREPARATION OF PLATE COUNT AGAR 17
3.3.2 PREPARATION OF MANNITOL SALT AGAR 17
3.3.3 PREPARATION OF MACCONKEY AGAR 17
3.4 SERIAL DILUTION 18
3.5 DETERMINATION OF TOTAL AEROBIC COUNT 18
3.5.1 SUB-CULTURING 18
3.6 BIOCHEMICAL TESTS 19
3.6.1 CATALASE TEST 19
3.6.2 CITRATE UTILIZATION TEST 19
3.6.3 GRAM STAINING AND MICROSCOPY 19
3.7 DETERMINATION OF STAPHYLOCOCCUS AUREUS 20
3.8 DETERMINATION OF TOTAL COLIFORM COUNT 20
3.9 DATA ANALYSIS 21
CHAPTER FOUR 21
RESULTS AND DISCUSSION 21
4.1 WATER ACTIVITY 21
4.2 MICROBIAL LEVEL OF „SHITO" SAMPLES 22
4.2.1 TOTAL AEROBIC COUNT (TAC) 23
4.2.3 QUALITATIVE ASSESSMENT OF COLIFORMS 28
4.3 QUALITATIVE ASSESSMENT OF MICROBIAL QUALITY OF SAMPLES 30
4.3.1 STAPHYLOCOCCUS SPECIES 30
CHAPTER FIVE 32
CONCLUSION AND RECOMMENDATION 32
5.1 CONCLUSION 32
5.2 RECOMMENDATION 32
REFERENCES 33
APPENDIX 37

LIST OF TABLES

TABLE 4.1: TOTAL AEROBIC COUNT ON „SHITO" SAMPLES FROM REGISTERED AND UNREGISTERED 26

CATERING SERVICE PROVIDERS IN KUMASI 26

TABLE 4.2: TOTAL COLIFORM COUNT ON FOOD SAMPLES FROM FORMAL AND INFORMAL 29

CATERING SERVICE PROVIDERS IN KUMASI 29

TABLE 4.4: MORPHOLOGICAL AND BIOCHEMICAL PROFILE OF COLIFORM ISOLATES FROM MA 31

PLATES 31

TABLE 4.5: QUALITATIVE ASSESSMENT OF *STAPHYLOCOCCUS* SPECIES IN FOOD SAMPLES FROM 33

FORMAL AND INFORMAL CATERING SERVICE PROVIDERS IN KUMASI 33

TABLE 4.6: MORPHOLOGICAL AND BIOCHEMICAL PROFILE OF COLIFORM ISOLATES FROM MA 33

PLATES 33

LIST OF FIGURES

FIGURE 3.1: QUANTIFICATION, ISOLATION AND IDENTIFICATION OF MICROORGANISMS..... 18

FIGURE 4.1: WATER ACTIVITY (A_W) FOR SHITO SAMPLES FROM FDA REGISTERED AND 23

UNREGISTERED CATERING SERVICE PROVIDERS IN KUMASI..... 23

FIGURE 4.2: TOTAL AEROBIC COUNT ON SHITO SAMPLES FROM (A) UNREGISTERED AND (B) .. 25

FIGURE 4.3: TOTAL COLIFORM COUNT ON FOOD SAMPLES FROM FORMAL AND INFORMAL 28

CATERING SERVICE PROVIDERS AFTER 48 HOURS INCUBATION AT 37^0C. 28

LIST OF PLATE

PLATE 4.1: COLIFORMS GROWTH ON MACCONKEY AGAR AFTER 24-HOUR INCUBATION AT 37^0C……. 31

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

One of the essential elements in foods is water, its impact cannot be underestimated. For some time now, checking of free water in ingredients or foods has become a necessity (Novasima, 2005). The underlying fact is that free water contributes to the growth of microorganisms. The impact of free water in ingredients or in foods enhances the growth of undesirable organism or other harmful substances (Banu 2002; Nicolau *et al*., 2006). The water activity (a_w) profile always comes into play as it enlightens us on some vital information pertaining to the likelihood of microbial growth on the surfaces of foods (Novasima, 2005). Water is an essential element in the facet of our lives, the activity of water significantly influences the growth of microorganisms in foods, the measured quantities or values of water in foods has a direct relation with microbial growth and other metabolic acts (Chirife and Buera, 1996).

The rate at which water functions in foods is influence efficiently or inefficiently by the activity of water. If water in foods were held with another element of molecules that underperforms in a chemical hydrolysis, the overall output of the water activity in the food would be dwindled. In most cases, the tightly bound water in foods has no inclination or disposition to evaporate and as a result, it exercises or apply minimal or no pressure and always result in water activity of zero (Sandulachi, 2012). There is a clear distinction between water activity and water content. Water activity can be used determine the storage, shelf life and stability of food or sauce such as the hot pepper

sauce. The activity of the water predicts the growth of microorganisms and influences the potentialities of food spoilage in a food sample (Sandulachi, 2012).

Food security and food stability mostly determined by (pH) levels. These levels are influenced by external environment and water activity of the food. It had been concluded that, water activity is higher with the perishable foods. Regardless of that, at low potential of hydrogen values and minimal water activity (a_w), highly soluble concentrations are mostly tolerated by species such as moulds. These species unfold a lot of risks on food stability and security especially foods with intermediate moisture content (Banu, 2002; Nicolau, 2006; Powitz, 2007).

For microorganisms to thrive in food products, it needs some appreciable water before the microorganisms can grow in food products. There is a required amount of water that promotes the growth of microorganisms; microbiologists have certain determinants or requirements that facilitate the growth of microorganisms in the shape of water activity (Rahman 2010). The emergencies of metabolites and other microbial contents are sometimes bent on or incline to alterations or changes in water activities. Environmental factors in some cases influence the levels of microorganisms with emphasize on water activity; the maximum and minimum rate of microorganisms depends on some environmental growth factors. Taxonomy by classification is a key factor in the environmental response of microbial growth (ICMSF, 2001).

Generally, the simple carbohydrates such as raw sugar, brown sugar, corn syrup, glucose etc are the first used nutrients before the complex nutrients are utilized. The level of development and growth is sometimes determined by the nutritional content of that

particular food. High level of nutrients in food products is a clear causative agent for the growth of pathogens (Darko, 2016).

Temperature plays an integral role in the development and growth of microorganisms; the internal and external factors, coupled with other technological factors influence the growth of microorganisms and its related pathogens. Biologically, there are some organisms which use flora in foods to influence pathogenic species (ICMSF 1980). Environmentally, organisms grow in foods in a unique manner, with a unique rate of growth; the presence of genes also affects the cells count, lag times and generational times. The conglomeration of metabolism mostly limits the thriveness of pathogens or species. The continuous interplay that exists between species or microorganisms and other related environmental components or elements may give rise to associational flora; there is an interaction or interplay between association and succession of flora in microbial and it profile contents (ICMSF 1980).

In Ghana, „shito" or black hot pepper sauce can be multi-faceted in the food service industry or domestically; it mostly serves as condiments; it can be used with other foods. Ghanaians have developed a great taste for black pepper sauce "shito". Most of the hospitality industries in Ghana prefer "shito" to hot oil; as they are all condiments (FAO, 2016). Hot sauce is used both in amping up a recipe during the cooking process, or as a condiment to top anything from scrambled eggs to stir-fry to fresh-baked bread to finish with fiery kick (Seis, 2018). The nutritive value of „shito" or blacked hot pepper sauce comprises of sodium and potassium. In one tablespoon of shito food, the body gets 190grams of sodium and 14grams of potassium. This sauce is very good for slimmers because it does not increase the amount of fats in the body. If you feel the need to eat healthy and lose weight „shito" is a way forward (Gracia, 2018).

1.2 Statement of the Problem

Hot pepper sauce “shito” is one of the commonly used products in our homes, schools, restaurants etc, that sometimes serves as condiments. The hot pepper sauce “shito” has gained popularity and is mostly patronized by students, food vendors etc. Our preference for hot pepper sauce has been increasing daily, and it will be expedient to consider the production, safety, water activity, microbial safety of this food product. Moreover, for health reasons, shelf life of the product and other pertinent concerns in our food industry, it is always a worthwhile to take proper measures in our preparation of condiments such as hot pepper sauce (Ref). There is a research gap in the safety of hot pepper sauce “shito”. The researcher thought it expedient to study the water activity profile and the microbial safety of hot pepper sauce “shito”.

This study therefore seeks to determine the water activity profile and microbial safety of hot pepper sauce „shito“. The determinants were S*taphylococcus* bacteria, salmonella bacteria, C*lostridium perfringens* in the hot pepper sauce and the water activity profile of the hot pepper sauce.

1.3 Objective

- To determine the water activity profile and microbial safety of hot pepper sauce „shito“.

1.4 Significance of the Study

The high patronage of hot pepper sauce “shito” among consumers or customers in Ghana has given us a course for concern. In promoting a healthy living with respect to what we consume, production losses, stability, food security, etc the need has come to ascertain the water activity profile and microbial safety of registered and unregistered hot pepper sauce „shito“.

It is hoped that the findings of the study, will be of immense help to stakeholders such as the „shito" producers, restaurants operators, the Food and Drugs Authority (FDA), Ghana Standard Authority, Ministry of Health (MOH), Ghana Health Service (GHS) and other institutions who may have interest in the outcome of the research. This research finding will form a background for researchers who will be conducting research in a similar area. Consumers will be abreast with the impact of water activity and microbial safety of hot pepper sauce „shito".

1.5 Limitations of the Study

The challenges encountered at the course of this thesis were related to inadequacy of resources being it time and other accessibilities. Financial constraints had a toe on the sample size for the study; few samples were used because of the financial challenges. Some of the „shito" producers fell reluctant to respond to questions. The scope of the study covered only the water activity profile and microbial safety of hot pepper sauce „shito", the impact of water activity on microbial growth of shito and the impact of water activity level and microbial load on food safety and shelf life of „shito".

CHAPTER TWO

LITERATURE REVIEW

2.1 The concept of water activity profile

Water is an essential element in the facet of our lives, the activity of water is very instrumental in the development and growth of microorganisms, the measured quantities or values of water in foods has direct relations with microbial growth and other metabolic acts (Chirife and Buera, 1996 as cited in Sandulachi, 2012).

According to Sandulachi (2012), the activity of water influences the physical reactions in foods. Thus the rate at which water functions in foods either efficiently or inefficiently boils down to the activity of the water. If water in foods is held with another element of molecules that underperforms in a chemical hydrolysis, the overall output of the water activity in the food would be dwindled. In most cases, the tightly bound water in foods has no inclination or disposition to evaporate (Sandulachi, 2012). The reductions in the amount of water in foods inhibit the activities or growth of microorganisms (Adams and Moss, 2008).

There is a clear distinction between water activity and water content. Water activity can be used determine the storage, shelf life and stability of food or sauce such as the hot pepper sauce. The activity of the water predictsor forecasts the potentialities of microorganisms that can cause infections and spoilage of foods (Sandulachi, 2012d). The activity of water in an ideal solution does not depend on temperature to cause the growth of microorganisms. In practicality, the activity of water sometimes changes with respect to temperature ranges (Fontana, 2001). The energy conditions of water in a system are ascertained through the water activity. Measures have been outlined to help address the functioning of water activity. These measures or factors that help to curb the activity of water in a system consist of the capillary effect and the colligate effect (Ross, 1993). There are other factors, which also help reduce the energy of the water activity and its relative humidity. These factors

include osmotic effect and matrix effects (Fontana 2001). Water bindings or the state of matrix can cause temperature to change the activity of water. The effect of temperature on the water activity of a food varies according to that particular product.

Scott (1953 as cited in meter food, 2018) believed that, water content in foods do not influence microbial load but the water activity in foods rather influence the development and growth of microorganisms. Water activity is highly considered by stakeholders such as producers of foods, to be the major means by which pathogens and microorganisms proliferate or multiplies. In the works of Scot, he considered the microbial proliferation or growth in dried foods, powered and mixes drinks, non-food products etc (Powitz, 2007).

2.2 The impact of water activity on the shelf life or stability of foods

Water activity mostly determines the stability and shelf life of food products, unlike water content. The activities of water in some cases identify and predict the microorganisms that show potentialities of food spoilage. In maintaining the physical and chemical stableness of foods, the water activity plays an integral role in the maintenance of food chemical stabilities (Fontana, 2001; utm.md). Apart from water activity, other ideal conditions of temperature and humidity also affect the stability and shelf life of food products. Shelf stability basically explains how food products are able to stand the test of time, but it again has a rippling effect on food conditions such as texture, mouth feel, moisture, clumping etc (Powitz, 2007; Fontana, 2001). In another context, the stability of food and its security is liable on activity of water and potential of hydrogen in the environment where the foods are found. It had been concluded that, water activity is higher with the perishable foods. Regardless of that, at low potential of hydrogen and minimal water activity (a_w), these species unfold a lot of risks on food stability and security especially foods with intermediate moisture content (Banu, 2002; Nicolau, 2006; Powitz, 2007).

There are different methods for food storage and food security or stability. However, product stability or storage without refrigeration in our homes and shops is a matter of concern for both developing countries and developed or industrialized countries (Leistner, 2012). Leistner further emphasized that, advanced countries preferred shelf products that are stable to moisture intermediate foods; well advance countries see shelf products as lively and attractive (FAO 2010; utm.md).

2.3 The role of water activity in ensuring quality and food safety

The activity of water in foods has become a major concern for most stakeholders in the food service Industry. The role been played by water activity cannot be overemphasized because of it critical nature. Some research works have proved that, the mode of controlling the activity of water in food can be curbed through drying of the, by using the freezing method, by using the salting method; these proven methods fight against food spoilage (Fontana 2001). The actions of the activity of water promotes the growth of microorganisms and potentially acts as chemical and biochemical reactions depending on the energy state of water in the food.

2.4 Factors that influence microbial safety in food products

A microbial activity does not operate in a vacuum. There are enormous factors that come into play in the determination of microbial activity. The microbial activity and microbial safety in foods will be reviewed based on, internal or micro factors (moisture content, potential of hydrogen pH and acidity, nutrient content, biological structure) and external or macro factors (temperature, processing steps, packaging, storage, presence of oxygen)

2.4.1 Micro or internal factors

Moisture content

For microorganisms to thrive or survive in foods, it needs some appreciable amount of water. Experts in microbiology appreciate the fact that, the requirements basically involve the activity of the water and the immediate surroundings or environment of the food (Jay, 2002). The activity of water always creates room for microorganisms to survive because it necessitates various forms of reactions such as chemical and biochemical (Mossel *et al.*, 1995). Microorganisms fully develop and growth fresh related foods because of high water activity values. The manipulation or control of water activities in food can be engineered by physical and chemical methods; where the physical method involves salting, drying, freezing and the chemical method focus on the binding of the water (Ghaly, 20100). The response of microorganisms differs proportionately, the presence of metabolites, water activities give rise to different or changes in microorganisms‟ response (Mossel *et al.*, 1995;).

Potential of Hydrogen (pH) and Acidity

Potential of hydrogen (pH) and acidity are sometimes used as a control mechanism of microorganisms. Pathogens and microorganisms can either thrive in foods at potential of hydrogen (pH) level below their minimum growth. Sometimes, pathogens do not survive at low levels of potential of hydrogen (pH); there is an exception, few can survive with low (pH). The buffering capacity of a food serves as a resistance to changes in potential of hydrogen (pH). Less buffering ability or capability of food products influence pH values in consonance with acidic compounds (Enser, 2001).

Nutrients content

The growth of microorganisms in foods is sometimes determined by the nutrients in the food. The exposure of some vital nutrients in foods limits the rapid development and growth of pathogens and other microorganisms. In another breath, the exposure of nutrients in foods serves as a fertile ground for microorganisms to multiply or growth (Prescott, 2002). For microorganisms to function efficiently, they will need some required nutrients in its operations or activities. Specifically, each microorganism thrives on different nutrients and ranges (Prescott, 2002).

Biological structure

There are physical barriers in animal and plant foods that prevent the growth of microorganisms. The composition of the biological structure serves as a safeguard against the growth of pathogenic organisms. There are some influencing factors that can cause microorganisms to penetrateinto these foods. “The maturity of plant foods will influence the effectiveness of the protective barriers and physical damage due to handling during harvest, transport, or storage, as well as invasion of insects can allow the penetration of microorganisms” (HPA, 2001). In preparing of food, the processes and activities involve can annihilate the physical barriers that prevent the entry of microorganisms. The processes sometimes make the foods contaminated and this can influence the growth of microorganisms.

2.4.2 Macro or external factors

Types of packaging

The atmosphere is full of gases and some of these gases have direct toxic effect that inhibits microbial growth. There are other gases that exhibit inhibitory mechanism through modifying the gas composition and these gas compositions indirectly alters the ecology of

the microbial environment. Technologically, measures have been put in place to curb the development and growth of pathogens. These measures include the method of modernizing the atmospheric form of packaging, the method of storage through atmospheric control (Loss and Hotchkiss, 2001).

Temperature conditions

There is always a correlation between temperature and microbial load or growth. Time and temperature have a direct impact on the rate of microbial pathogens. The longevity or the shelf life of product will be determined by the extent of microbial load. In determining the shelf life of a product, an emphasis will be laid on the microbiological safety of the product (Phimolsiripol and Suppakul, 2016). Every pathogen and microorganism has certain specified range of temperature that they thrive. The correlation of temperature related conditions and the rate of development and growth of microorganisms show dynamism among the individual microorganisms (Darko, 2016). According to Darko (2016), the maximum temperature for pathogenic microorganisms to produce occurs between 14 °C to 40 °C, however, a few microorganisms have survival rate below 0 °C.

Storage conditions

The factors to be considered in the storage condition are the temperature related factors specifically the holding factor, the time related factors and conditions after cooking, the wetness "humidity" of the cooked food and the effectiveness of the packaging materials (HPA, 2009). Some of the most influencing factors that cause the growth of microbial pathogens are time and temperature; these factors determine the storage life of refrigerated foods (Prescott, 2002). The interconnection between the contributing factors, thus the intrinsic and the extrinsic is very pertinent to selecting a proper storage facility or condition for food products.

Presence of Oxygen

The survival rate of aerobic and anaerobic microorganisms is determined based on their requirement of oxygen (Jay, 2002). According to Hentges (1996), pathogens or bacteria have the propensity to grow or survive aerobically but also have the capacity to thrive anaerobically if oxygen is not present. The major requirement of life is basically water and oxygen, "the minimum permissive oxygen concentration and the maximum permissive oxygen concentration are, respectively, the lowest and the highest oxygen levels that the organism will tolerate" (Lumenlearning, 2018). The support of aerobic growth in cells is been necessitated by oxygen. According to Kazzaz (1996) as cited in Baez and Shiloach, 2014), the essence of oxygen towards growth and production cannot be under estimate, the oxygen is also known to be toxic at high concentrations for a variety of cell types.

Processing steps

The Scientific means for deciding whether food safety can be determined by time or temperature should consider the processes that destroy vegetative cells, the conditions associated with packaging, issues of handling after processing prevents the introduction of vegetative forms of pathogens; some packaging instruments and equipment help prevent the resurfacing of microorganisms into the food (Jay 2002).

2.5 Microorganisms in Food

The propensity of food having microorganisms is very high. Bacteria, viruses, fungi, protozoa etc are some examples of microbes. Foods are being characterized with microorganisms such fungi and bacteria and to some extent protozoa and viruses.

Bacteria

Comparatively, the size of bacteria is much larger than viruses (DiMaio, 2012). Bacteria can be in the form of *Salmonella*, *Staphylococcus, Listeria monocytogenes, Escherichia coli*, *Shigella*, *Clostridium* etc (Prescott, 2002). According to Mariott and Gravani (2006) some bacteria helps in locomotion whilst others resort to pigmentation. Jay (2002) asserted that, some bacteria produce pigments that sometimes alter the colour of food. The impact of bacteria cannot be overemphasized, some of the bacteria are very detrimental to human health and others are also of importance or beneficial to the human system.

Fungi

Fungi as a microorganism can be in different forms. An example of fungi includes; yeast, mushrooms, moulds. The uses of fungi are quite enormous; it can be a pathogen and act as causative agent of diseases and infections (Prescott, 2002).

In most cases, food spoilage happens or occurs through moulds (Mariott and Gravani, 2006). Fungi can serve as a source of antibiotic; it can serve as a fruiting agent as in the case of mushrooms. There are fungi diseases, which adversely affects both humans and animals. There will an adverse repercussion on food supplies, health, the economy etc if fungi degenerate.

Viruses

In virus to bacteria, virus is smaller in size than bacteria. Virus comprises of proteins and other genetic materials. Viruses cannot replicate in foods, the main means of transmission of virus is food vendors, handlers and the use of unhygienic equipments can transfer virus to foods (DiMaio, 2012). According to Darko (2016), for viruses to replicate or multiply, it needs the assistance of other organisms. Food detected viruses are often infested or transmitted by either those serving the food or the carriers of the food (Prescott, 2002). If the basic required sanitary practices are not ensured, the issue of food infestations will be

very high. Those who come into direct contact with foods in the course of preparation must observe the basic cooking etiquette. Issues of viral infections are on the rise; food handlers must be properly screen to ascertain if they do not have any health complications.

2.5.1 Microbial quality of condiments

Hot pepper sauce „shito" is classified as a condiment because it enhances and complements food. The production process and the handling of the already made product come with a lot of risks. They are often produced in mass quantities and may be stored for use for period of time, which may not be in a temperature control (Reynolds *et al*., 2010).

2.6 The concept of hot pepper sauce „shito"

Hot black pepper sauce popularly called „Shito" in the Ghanaian parlance is one of the desirable sauce in the Ghanaian cuisine (Wikipedia, 2018). According to Gracia (2018), the nutritive value of shito comprises of sodium and potassium. The makeup of shito includes different ingredients depending on how you want the taste. Shito is sometimes used as a condiment in our Ghanaian dishes. Domestically, hot pepper sauce "shito" serves as condiments for numerous foods. In most restaurants across Ghana, shito replaces a lot of sauces as a condiment to fried rice or steamed rice (Wikipedia, 2018). The taste, flavour, texture, stability and the heat level of the sauce is determined by the production process or the brand (Bonappetit, 2008).

2.7 Microbial load of Foods sold in shops and on streets in Ghana

Efforts must be made to look into the microbial safety of foods sold in our vicinities and on our streets. Major stakeholders and authorities should monitor our environmental sanitations at places where food are bought and served. Stakeholders should support

institutions such as the Food and Drugs Authority, Ghana Standard Authority, in the pursuance of implementing systems and measures to control foods. The industry players and other major stakeholders are always on alert to improve on their products to avoid any unfortunate circumstances (Mensah *et al*., 2002; scielosp.org). There are a lot of dangers or hazards associated with street foods. Food vendors on the street should be given refresher courses and other orientation programmes to update them on current issues pertaining to their field.

2.8 Sources of food contamination

Microbial activities thrive on contaminated foods and sometimes the microbial activity get foods contaminated as well. The sources or cause of food contamination are generally given as; poor hygienic practices, contaminated cooking equipment or materials, foods poorly cooked or inadequate cooking, food from unsafe sources etc. According to Donkor *et al.*, (2008) as cited in Darko, (2016) contamination of food can be ascribed to some risk factors such as inappropriate and unhygienic cooking methods and practices. According to Mariott and Gravani (2006), the first point of call in the sensitization process of fighting against food contamination is how often and well food handlers wash their hands. A pertinent point to consider in the contamination of food and the transmission of pathogens is the serving stage of the food (Mensah *et al*. 2002).

CHAPTER THREE

METHODOLOGY

3.1 Materials

The agars used were products of OXOID Laboratories, Basingstoke Hampshire, England. They included Plate Count Agar used for the isolation of total viable count, Mannitol Salt Agar for isolation of *Staphylococcus* and MacConkey agar for total Coliform count. VWR Incubator (USA) and AquaLab Dew Water activity meter (USA) were used to incubate the inoculated agars and water activity of the samples used for the experiments.

3.2 Source of Sample

"Shito" samples were purchased from the major shopping centers in the Kumasi Metropolis. The areas are the Kumasi City Mall, Poku Trading, Asafo Market, Central Market and the Ayigya Market. Samples purchased were further groups into those registered with the Food and Drugs Authority and those that are not. This grouping was done using a list obtained from the Food and Drug Office.

3.1 Water Activity Determination

Samples were homogenized and placed in the disposable sample cup to completely cover the bottom of the cup (half full). Excess sample on the rim and outside of sample cup were cleaned with a clean „Kimwipe". The sample cup was then placed in the sample chamber and the knob turned into the "Read" position. The water activity and temperature was recorded after the reading had stabilized and the display no longer in the read mode. Distilled water was used as a quality control check prior to the start of sample analysis.

3.2 Sample Preparation

The process carried out in quantifying, isolating and identifying the microorganismsis summarized in a block diagram as shown below

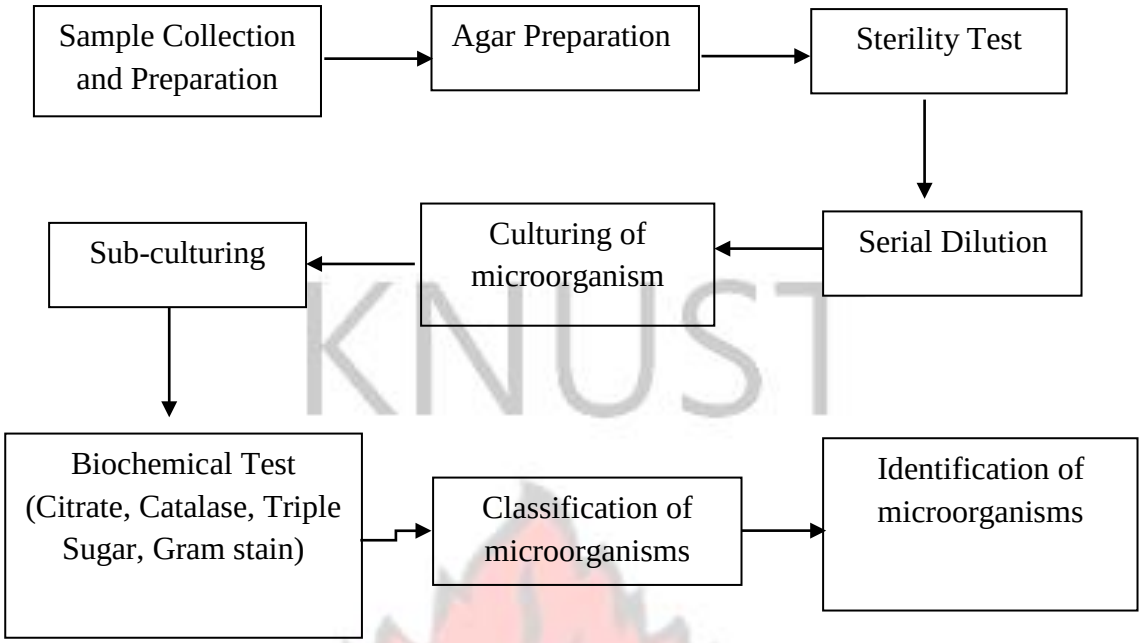

Figure 3.1: Quantification, Isolation and Identification of Microorganisms

3.3.1 Preparation of Plate Count Agar

Plate Count Agar (Nutrient agar) was prepared by suspending 23.5 grams in 1000 mL (1 liter) distilled water and heated to boil to dissolve completely. It was sterilized at 121°C for 15 minutes in sealed bottle. The sterilized agar was left to cool at 50°C before pouring into sterile Petri plates.

3.3.2 Preparation of Mannitol Salt Agar

Agar powder 111 g was suspended in 1 liter of distilled water and brought to boil to dissolve completely. It was sterilized by autoclaving at 121°C for 15 minutes.

3.3.3 Preparation of MacConkey agar

Agar powder (26 g) was suspended in 500ml of distilled water and brought to boil to dissolve completely. It was sterilized by autoclaving at 121°C for 15 minutes and allowed to cool to 50°C and poured into sterile Petri dishes.

3.4 Serial dilution

Serial dilution was done to reduce a dense culture of cells to a more usable concentration. Each dilution done reduces the concentration by a certain amount. A mass of 5g of each sample were weighed and placed in 45ml of peptone water solution. Serial dilution was performed for 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, $10^{-5}$and 10^{-6}. The serial dilution done for 10^{-3}provided very clear and distinct isolate, hence it was selected for the subsequent analysis (Fung, 1985).

3.5 Determination of Total Aerobic Count

Spread plate technique was used to inoculate the microorganisms. An inoculum volume of 0.1ml from each dilution series was pipetted to their respective labeled petri dishes of plate count agar plates. The glass spreader (hockey stick) was sterilized using ethanol and spread over a bunsen burner. The solution was spread evenly over the agar plate using the hockey stick while carefully rotating the petri dishes underneath an angle of 45^0. The Petri dish now containing the agar and evenly distributed sample solution was incubated at 37^0C for 24hours. The colonies of microbes that appeared were enumerated and recorded (Fung, 1985).

3.5.1 Sub-culturing

The sub-culturing is done to transfer cultures from one medium by inoculating into another medium. It was done to isolate microorganisms from a mixed culture to obtain a pure culture using streak plating. Single colonies of the microorganisms identified as unique isolates upon morphological basis were picked and streaked unto fresh nutrient agar plates and incubated for 24 hours to obtain pure isolates for further testing (Fung, 1985).

3.6 Biochemical Tests

The Biochemical tests were done to confirm the identities of the isolated microorganisms and also to predict their fermentative profiles and prowess. The tests considered in this study included the catalase, triple sugar iron (TSI), citrate and Gram staining.

3.6.1 Catalase Test

The catalase test is done to distinguish among Gram-positive *cocci* members. Catalase test is also used to differentiate aero tolerant strains of clostridium, which are catalase negative, and bacillus, which is catalase positive (Fung, 1985). A volume of 5ml of 3% H_2O_2 was prepared. A drop of 3% H_2O_2 was placed in a glass slide. A loop was used to transfer a small amount of colony growth in the surface of a clean, dry glass slide

3.6.2 Citrate Utilization Test

Simmons citrate test probes the ability of microorganisms to utilize citrate as their carbon source. Organisms, which can utilize citrate as their sole carbon source, use the enzymes citrase to transport citrate into the cell. A sterilized straight inoculation needle was used to touch the top of a well-isolated colony. Simmons citrate agar was inoculated on the slant through the center to the bottom and then streaking unto the agar slant. Agar slant was incubated at 35^0C.

3.6.3 Gram staining and Microscopy

Gram stain is used to distinguish two large group of bacteria based their different cell wall constituents. The gram stain distinguishes between gram positive and gram negative groups by coloring these cells red or violet. A small amount of well isolated colony was placed in a drop of physiological saline water on a dry slide. The slide was flamed to kill

microorganisms and fix unto the slide. Crystal violet staining reagent was placed on the heat fixed cell for about 1 minute. The slide was gently washed with distilled water. Gram A mordant, gram iodine was placed on slide and washed alcohol used as a decolorizing agent. Safranin was used as a counter stain and placed on the side then washed with distilled water until color disappears. A drop of immersion oil was placed on each slide to observe it under microscope (Fung, 1985).

3.7 Determination of *Staphylococcus aureus*

Staphylococcus species were isolated and enumerated by spread plate method and grown on Salt Mannitol Agar (SMA). Serial dilutions of 10^{-1} to 10^{-4} were prepared by diluting 10 g of sample into 90 ml of sterilized peptone water for stock dilution. One milliliter aliquots from each of the dilution were inoculated into Petri dishes with already prepared SMA. The inoculum was evenly spread with a sterile bent rod and allowed to dry for 15 minutes at room temperature. The plates were inverted and incubated at 35 ºC for 24 hours. After incubation yellow colonies were counted and recorded as *Staphylococcus* counts.

3.8 Determination of Total Coliform Count

The TCC was carried out by spread plate method on MacConkey agar (MA). Serial dilutions of 10^{-1} to 10^{-6} were prepared by diluting 10 g of sample into 90 ml of sterilized peptone water for the stock dilution. One milliliter aliquots from each of the dilution were inoculated into Petri dishes with already prepared SMA. The inoculum was evenly spread with a sterile bent rod and allowed to dry for 15 minutes at room temperature. The plates were inverted and incubated at 35 ºC for 24 hours.

3.9 Data Analysis

Results are presented as graphs. Data was subjected to the T-test and ANOVA to test for significant difference at 95% confidence interval using GraphPad Prism software 6.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Water Activity

Water Activity (a_w) for the samples are shown in Fig 2, the results for FDA registered were between 0.51 and 0.88 while those for product not registered with the FDA were between 0.52 and 0.95. Even though, a_w for FDA registered product were higher than those not registered with FDA, there were no significant difference observed ($p>0.05$).

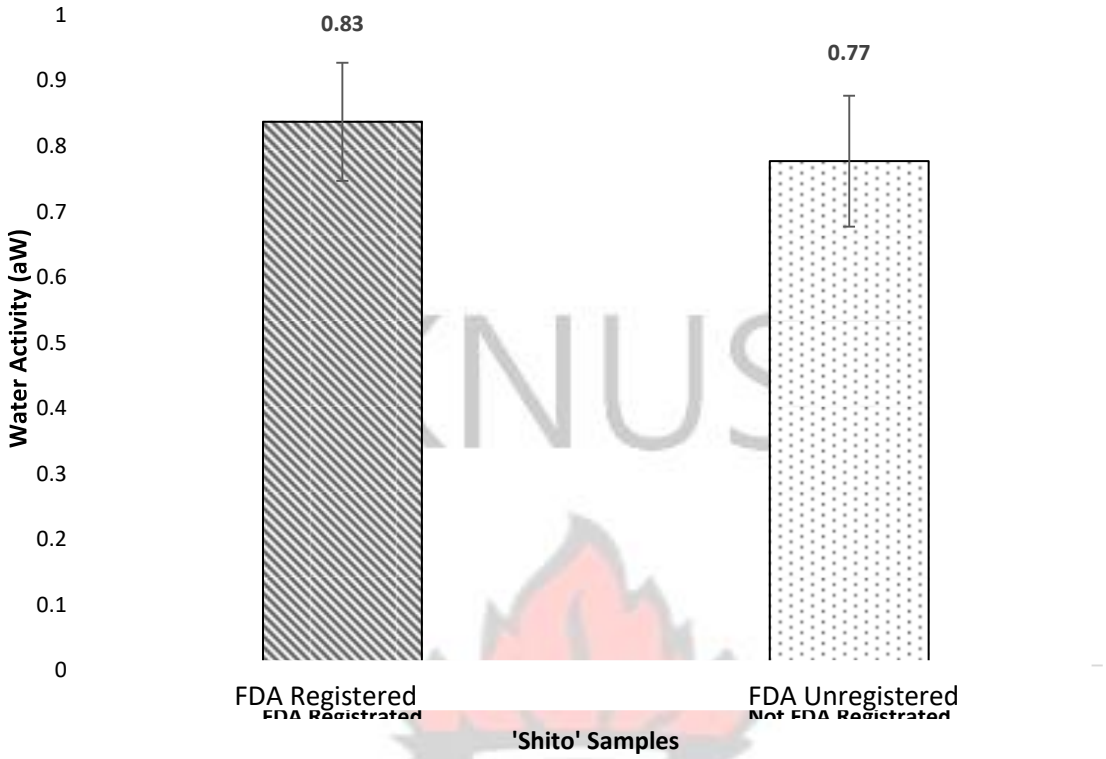

Figure 4.1: Water Activity (a_w) for Shito samples from FDA registered and unregistered catering service providers in Kumasi

According to Roos (1993), there is a non-linear relationship between moisture content and water activity is related to microbial spoilage. The minimum level of water activity influences the growth of different microbial organism. Bacteria were the most sensitive and below a_w of 0.90 are inhibited. However, at lower a_w or higher levels of solutes in water, moulds and yeasts are more tolerable and with a minimum a_w of 0.70 – 0.80 and 0.87 – 0.94 respectively can growth and proliferate. However, at less than 0.6 a_w, most microorganisms cannot growth.

4.2 Microbial Level of „Shito" Samples

The qualitative and quantitative assessment of microbial diversity of food is very crucial in the determining of food quality and safety. Some organisms exhibit pathogenicity at certain numerical thresholds thus their pathogenicity being an index of population whereas

others exhibit pathogenicity irrespective of their population (Giraffa, 2004) This unique feature of microorganisms warrants both quantitative and qualitative assessment in the establishing of food quality and safety. The results obtained from the quantitative study did render some of the samples unwholesome for consumption as ready-to-eat products with no further processing.

4.2.1 Total Aerobic Count (TAC)

The total aerobic count assay indicated the presence of some aerobic microorganisms in the samples with some exhibiting significant microbial populations though few. The registered samples generally recorded low microbial contaminations (23%) as opposed to the unregistered samples (46%).

The statistical computation of the outcome did show significant difference ($P<0.05$) in the counts obtained from the various samples at 95% confidence interval (CI) for both the registered and unregistered samples.

The results did show though 46% of the unregistered samples recorded microbial contaminations, only 23% exceeded the safe and acceptable limit of 1.0×10^4 cfu/g whereas only 7% of the registered samples exceeded the safe limit. This clearly establishes the relative safety of the registered samples as against the unregistered ones. The counts recorded are presented in table 3.0 in colony forming units (cfu/g) with their standard deviations.

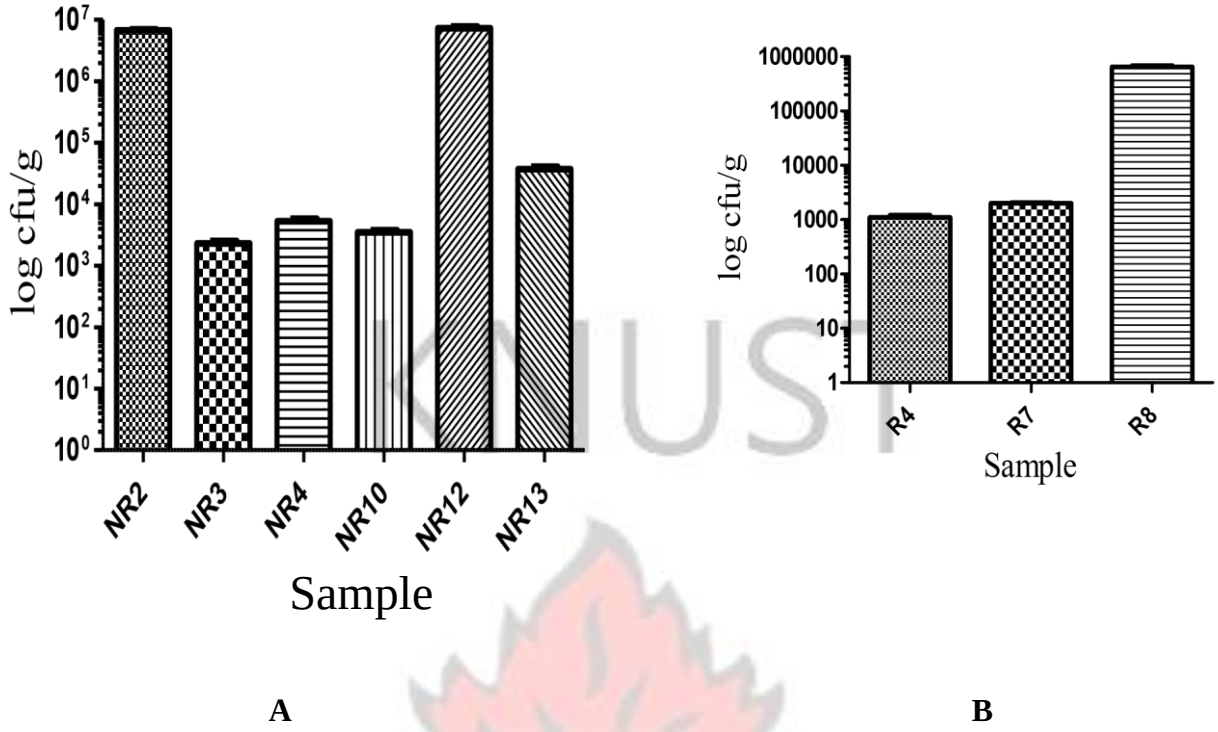

Figure 4.2: Total aerobic count on Shito samples from (A) Unregistered and (B)

Registered sources after 48 hours incubation at 37^0C.

Table 4.1: Total aerobic count on „shito" samples from registered and unregistered catering service providers in Kumasi

Analysis code	Registered (cfu/g)	Unregistered (cfu/g)	Reference
S1	<30	0.00 ± 0.00	1.0×10^4
S2	<30	6.7×10^6 ± 4.04	"
S3	0.00 ± 0.00	2.3×10^3 ± 4.00	"
S4	1.1×10^3 ± 2.00	5.3×10^3 ± 9.54	"
S5	0.00 ± 0.00	0.00 ± 0.00	"
S6	0.00 ± 0.00	<30	"
S7	2.2×10^3 ± 3.61	0.00 ± 0.00	"
S8	6.4×10^5 ± 6.65	0.00 ± 0.00	"
S9	0.00 ± 0.00	0.00 ± 0.00	"
S10	0.00 ± 0.00	3.5×10^3 ± 5.29	"
S11	<30	0.00 ± 0.00	"
S12	0.00 ± 0.00	7.3×10^6 ± 9.00	"
S13	0.00 ± 0.00	3.7×10^4 ± 5.69	"

The total aerobic count assay establishes the total population of aerobic microorganisms in the sample and per ISO demands; the set upper limit for acceptability is counts below 1.0×10^4cfu/g. The results showed only 23% of the samples to have failed to meet the standard safe limit, which is a good indicator of quality since less than a quarter of the samples failing. This seemingly good report could be attributed to the mode of preparation of the shito. This is because shito is prepared by prolonged heat treatment through hours of cooking at high temperatures, which is enough to kill the mesophillic microorganisms, found in everyday activities both pathogenic and non-pathogenic. The mode of cooking which involves more oil and less water through evaporation, results in decreased moisture levels and low water activity; thus rendering the product not favorable for the thriving of microorganisms.

The unregistered samples recorded the highest microbial contaminations; $7.3\times10^6 \pm 9.00$ with a 23% occurrence as opposed to the registered samples recording $6.4\times10^5 \pm 6.65$ with 7% occurrence. The relatively good performance of the registered samples could be attributed to the good practices adopted by the producers to ensure a quality product fit for registration and this goes to highlight the essence of monitoring and ensuring compliance with acceptable standards. The unregistered samples on the other hand are produced by sources with little or no supervision and charge and thus on the larger scale do not conform to any standards and checks allowing for the implementation of appalling modus operandi and sanitary practices which reflects in the quality of the products.

Food processing procedures that involve direct human interfaces tend to also be a significant source of food contamination. Poor sanitation and personal hygiene practices on the part of processors tend to affect food quality as studies have shown a direct impact of processing line on food quality. The practices adopted by the producers during post production and packaging could also introduce contaminants into the products which

hitherto were safe. Conditions such as the quality of bottles or packaging containers used, the equipments used in the transfer of product into packaging material and hygiene of the workers could all contribute to contamination and less quality products.

4.2.2 Determination of Total Coliform Count

The total coliform count assay recorded results that correlated with the total aerobic count on the merit of food safety where all samples that were assessed to be unsafe with counts exceeding the acceptable limit also recorded coliform counts exceeding the acceptable limit of 1.0×10^{1}cfu/g. The other samples recorded no coliform presence and the trend was similar with that of the aerobic count with 7% coliform occurrence for the registered samples and 23% for the unregistered samples.

The statistical analysis inferred a significant difference between the counts from the two sources at $P<0.05$.

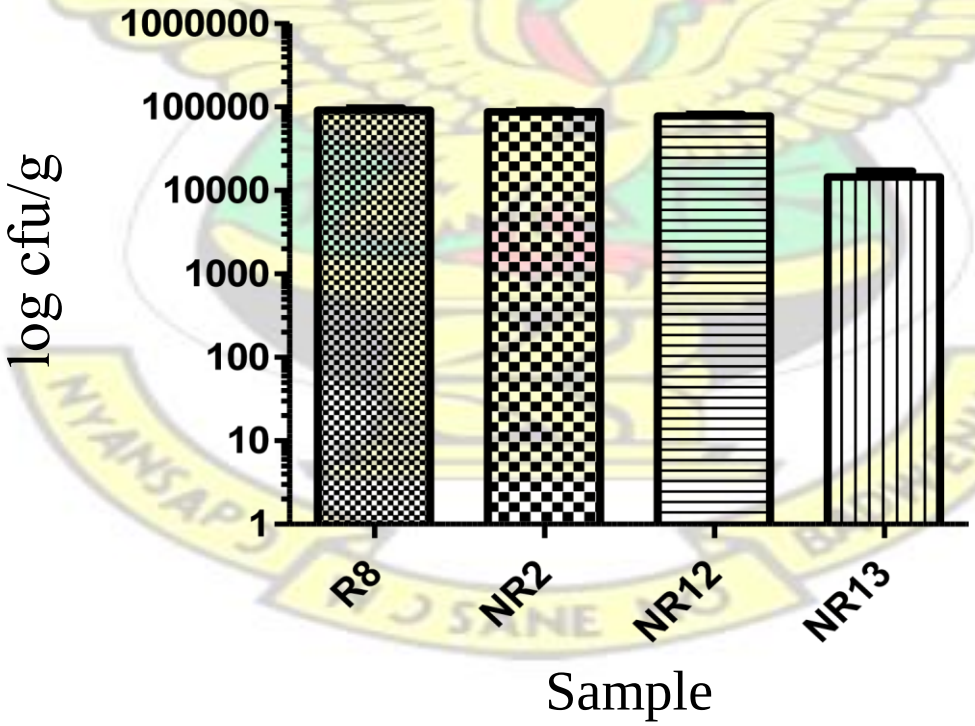

Figure 4.3: Total Coliform Count on food samples from formal and informal catering service providers after 48 hours incubation at 37^{0}C.

Table 4.2: Total Coliform count on food samples from formal and informal catering service providers in Kumasi

Analysis code	Registered (cfu/g)	Unregistered (cfu/g)	Reference
S1	0.00 ± 0.00	0.00 ± 0.00	1.0×10^4
S2	0.00 ± 0.00	8.4×10^5 ± 6.03	"
S3	0.00 ± 0.00	0.00 ± 0.00	"
S4	0.00 ± 0.00	0.00 ± 0.00	"
S5	0.00 ± 0.00	0.00 ± 0.00	"
S6	9.1×10^4 ± 5.51	0.00 ± 0.00	"
S7	0.00 ± 0.00	0.00 ± 0.00	"
S8	0.00 ± 0.00	0.00 ± 0.00	"
S9	0.00 ± 0.00	0.00 ± 0.00	"
S10	0.00 ± 0.00	0.00 ± 0.00	"
S11	0.00 ± 0.00	0.00 ± 0.00	"
S12	0.00 ± 0.00	7.5×10^5 ± 7.00	"
S13	0.00 ± 0.00	1.5×10^3 ± 3.79	"

The results did show a direct correlation between the TAC and Coliform assay where all samples that failed the TAC assay also recorded high Coliform count exceeding the acceptable limit of 1.0×10^1cfu/g. This observation highlights the relationship between hygiene and food quality as the samples that were adjudged to be of poor microbial quality per the presence of aerobic microorganisms also recorded high *E.coli* presence, which is an indicator of poor hygienic practices.

This also goes to suggest the contamination could have been post-production contamination particularly during the packaging and handling sections which involves direct human interfaces.

Coliforms are a class of organisms, which are of prime importance in the area of food safety as they have been implicated in a couple of food poisoning and contamination cases recorded across the globe. This makes Coliforms pathogenic and of concern to health and safety of consumers of meat. The family of Coliforms consists of organisms such as *Salmonella* spp. particularly the *S.typhi* which is the causative organism of typhoid which is one of the food borne illnesses very difficult to treat (Thiruvengadam *et al*., 1973). Other Coliforms are *Klebsiella pneumoniae* which causes pneumonia, *Enterococci* spp., and the infamous *Escherichia coli*responsible for diarrhea experienced from food contamination. These causes most countries to have a sharp eye for coliforms in foods with the standards ranging from 0 to 10 cfu/g as the tolerable limits but in
Ghana the coliform must be zero(0).

4.2.3 Qualitative assessment of Coliforms

The various coliforms identified were characterized based on their morphology on the agar plate via parameters such as form, color and surface descriptions as well as some biochemical properties such as gram reaction, oxidase and catalase reactions. The morphological characterization on the agar plates indicated the presence of some *Escherichia coli*. and some suspected *Enterococci faecalis* spp. were observed on the agar plates using the unaided eye but no *Salmonella* spp. were detected in the samples.

Plate 4.1: Coliforms growth on MacConkey agar after 24-hour incubation at 37^0C

All isolates identified are only suspected to be their respective identities as not sufficient biochemical tests were carried out to validate the identities with supporting morphologies.

Table 4.4: Morphological and Biochemical profile of Coliform isolates from MA plates

Isolate	Morphology	Oxidase	Catalase	Indole	Gas	Gram xtics*	Inference
2	Red colony	-	+	+	-	-rods	*E. coli*
3	Pink colony	-	+	-	-	-rods	*E. faecalis*

*xtics- characteristics

One typical Coliform that is of significance in food safety is *Escherichia coli* (*E. coli*) and is mostly used as the indicator for faecal contamination and human induced contamination. The detection of *E. coli* strains particularly the pathogenic strains in food is deemed a high alert factor thus the acceptable or tolerable limit of *E. coli* being set at 0cfu/g by the ISO and AOAC. The detection of high Coliform population and *E. coli* in the samples is an indication of poor sanitary and personal hygiene practices. *E. coli* in

particular is a faecal coliform and thus is easily sourced from improper hand washing after using the washroom.

4.3 Qualitative assessment of Microbial Quality of Samples

The numerical index of microbial quality is not enough in establishing food safety and thus requires some qualitative assessments. The identities of the detected microorganisms were established on the merit of both morphological and biochemical characteristics.

4.3.1 Staphylococcus species

The identification of Staphylococcus species was indexed on both morphological descriptions on the agar plates as well as biochemical profiles of the isolated suspected colonies on Mannitol Salt agar. The assay indicated the presence of two species being *Staphylococcus aureus* and *Staphylococcus epidermidis* distributed unevenly across the samples. The trend observed in the *Staphylococcus* assay is at part with that of the Coliform and TAC assay.

Table 4.5: Qualitative assessment of *Staphylococcus* species in food samples from formal and informal catering service providers in Kumasi

Analysis code	Registered (cfu/g)	unregistered (cfu/g)
S1	None detected	None detected
S2	None detected	Detected
S3	None detected	None detected
S4	None detected	None detected
S5	None detected	None detected
S6	Detected	None detected
S7	Detected	None detected
S8	None detected	None detected

S9	None detected	None detected
S10	None detected	None detected
S11	None detected	None detected
S12	None detected	Detected
S13	None detected	Detected

The colony morphology and biochemical characteristics of the isolates are presented in table 5.0 below.

Table 4.6: Morphological and Biochemical profile of Coliform isolates from MA plates

Isolate	Morphology	Oxidase	Catalase	Indole	Gas	Gram xtics*	Inference
1	Pink colony	-	+	-	+	+cocci	*S.epidermidis*
2	Yellow colony	-	+	-	-	+cocci	*S.aureus*

*xtics- characteristics

Staphylococcus aureus has been implicated as the causative organism of some clinical boils coupled with other symptoms such as high temperature, headaches and uneasy feelings (Liu *et al*., 2011). *S. aureus* is an indicator of environmental and human induced contamination as the organism is mostly found to inhabit living hosts such as man (Liu *et al*., 2011). This thus is not surprise as a correlation was observed between the Coliform count and *Staphylococcus* assay with the same sample batches falling culprit in all instances indicating bad hygienic practices particularly after cooking during packaging and handling.

The detection of *S.aureus* in the samples renders them unsafe for consumption according to the ISO regulations. This is due to the enormous health risks the consumer is exposed to upon consumption of *S.aureus* infested food (Liu *et al*., 2011).

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

FDA Registered shito samples recorded a significant difference lower total aerobic count (23%) as compared to unregistered samples (46%). The morphological characterization on the agar plates indicated the presence of *Escherichia coli*. and some suspected *Enterococci faecalis* spp. However, no *Salmonella* spp. was detected in the samples. A direct correlation was found between total aerobic count and coliform assay for samples, which failed the TAC assay. The presence of *E. coli* indicated the poor personal hygiene of the shito manufacturers. „Shito" produced by FDA registered caterers are safer and potential have longer shelf life due to their lower aW and microbial load as compared to non-FDA registered caterers.

5.2 Recommendation

There is the need for Food and Drugs Board to strength its post market surveillance and ensure registered "shito" manufacturers adhere to the regulations after obtaining their FDA license. Unregistered "shito" manufacturers should be encouraged to register with the FDA and educated on Good Manufacturing Practices to ensure food safety.

Personal hygiene is undoubtedly very essentially in human life. Is therefore expedient to promote and sensitize the public on the need of personal hygiene. The manufacturers of „shito" need to be circumspect in their operations because the presence of *E-coli* in the „shito" was attributed to poor hygiene on the part of the manufacturers.

REFERENCES

Adams, R.M and Moss, O.M (2008). Microorganisms and Food Materials. In: Adams, M. R. and

Moss. M. O (Eds) Food Microbiology. 3rd ed. Pp. 2-50. Royal Society of Chemistry Publishing. Cambridge.

Agbodaze, D., Nmai, N.P., Robertson, F., Yeboah-Manu, D., Owusu-Darko, K. and Addo,

K. (2005). Microbiological quality of khebab consumed in the Accra metropolis. *Ghana. Medical Journal*, 39: 45-50

Baez, A and Shiloach, J (2014). Effect of elevated oxygen concentration on bacteria, yeasts, and cells propagated for production of biological compounds. *Microbial Cell Factories* 13(181): 1 -7

Banu, C. (2002) Calitateaşicontrolulcalităţiiproduseloralimentare, Agir.Bucureşti. Pp.550

Barnes, M.J, and Taylor, B.M. (2012). Assessing food safety and associated food handling practices in the street food vending. Food Research Result.

Chirife, J. and Iglesias, H. A. (1996) Prediction of the effect of temperature on water sorption isotherms of food material, *Journal of Food Technology*, 11: 110-120

Darko, S. (2016). Microbiological Food Safety Standards Practiced by Some Hotel Industries in Kumasi. A thesis submitted to the Department of Food Science and Technology. Kwame Nkrumah University of Science and Technology. (Retrieved from Knustspace).

DiMaio, D (2012). Viruses, Masters at Downsizing. *Cell Host and Microbe*, 11 (6): 560561

Donkor, S.E., Lanyo, R., Akyeh, L.M, Kayang, B.B. and Quaye, J. (2008). Monitoring enterohaemorrhagic Escherichia coli O157: H7 in the vegetable food chain in Ghana. *Research Microbiology*, 3: 425-430.

Enser, M. (2001). Muscle lipids and meat quality. Biological Society of Animal Scence. Meeting proceedings. pp: 243-246. http://www.bsas.org.uk/ downloads/ annlproc/ Pdf20 01/243.pdf.

Fontana, A.J. (2001). Understanding the importance of water activity in food safety. *Cereal Foods World,* 45(1) : 8-12

Food and Agricultural Organization and World Health Organizations (FAO/WHO). (2010). Food Safety, Quality and Consumer Protection: Guidelines for Strenghening National Food Control Systems p 3. Joint FAO/WHO Publications

Food and Agriculture Organization of the United Nations (FAO) (2016). Street Food in Urban Ghana. A desktop review and analysis of findings and recommendations from existing literature. Edited by Marras, S and AgBendech Mohammed. http://www.fao.org/3/a-i5804e.pdf

Fung D.Y.C. (1985) Procedures and Methods for One-Day Analysis of Microbial Loads in Foods. In: Habernehl KO.(eds) Rapid Methods and Automation in Microbiology and Immunology. Springer, Berlin, Hedelberg. ISBN 978-3-64269945. pp 656-664

Ghaly, A.D., Dave, S. B and Brooks, M.S (2010). Fish Spoilage Mechanisms and Preservation Techniques: Review. *American Journal of Applied Sciences* 7 (7): 859-877

Giraffa, G (2004). Studying the dynamics of microbial populations during food fermentation. FEMS Microbiology Reviews, 28: 251–260

Health Protection Agency (HPA) (2009). Guidelines for Assessing the Microbiological Safety of Ready-to-Eat Foods. London: Health Protection Agency. http://webarchive.nationalarchives.gov.uk/20140714111812/http://www.hpa.org.uk/webc/HPAwebFile/HPAweb_C/1259151921557. Accessed on November 8, 2018

Hentges, D.J (1996). Anaerobes: General Characteristics. In: Baron S, editor. Medical Microbiology. 4th edition. Galveston (TX): University of Texas Medical Branch at Galveston; Chapter 17.Available from:

https://www.ncbi.nlm.nih.gov/books/NBK7638/

International Commission on Microbiological Specification for Foods (ICMSF) (1980). Microbial ecology of foods. Volume 1, Factors affecting life and death of microorganisms. Orlando: Academic Press . p 311.

Jay, J. M. (2002). Microorganisms in food. In: Jay, J. M. (Eds). Modern food microbiology, 6th ed. Pp 57-170. Aspen Publishers Inc. Gaithersburg, Maryland.

Leistner, L., and Gould G.W. (2012). Hurdle Technologies, Combination Treatments for Food Stability, Safety and Quality, New York, Kluwer Academic/Plenum Publishers.

Liu, C., Bayer, A., Cosgrove, S. E., Daum, R. S., Fridkin, S. K., Gorwitz, R. J and Rybak, M. J. (2011). Clinical practice guidelines by the Infectious Diseases Society of America for the treatment of methicillin-resistant Staphylococcus aureus infections in adults and children. *Clinical infectious diseases*, ciq146.

Loss, C., and Hotchkiss, J. (2001). Inhibition of Microbial Growth by Low-Pressure and Ambient Pressure Gases. New York, NY: Marcel Dekker

Lumenlearning (2018). Oxygen Requirements for Microbial Growth. https://courses.lumenlearning.com/microbiology/chapter/oxygen-requirementsfor-microbial-growth/ Accessed on November 1, 2018

Mariott, N. G. and Gravani, R. B. (2006). Personal Hygiene and Sanitary Food Handling. In Mariott, N. G. and Gravani, R. B. (Eds) Principles of Food Sanitation, 77-97 5th ed. Springer Science Business Media, Inc. New York. NY

Mensah, P. Yeboah-Manu, D., Owusu-Darko, K. and Ablordey, A. (2002). Street foods in Accra, Ghana: how safe are they? Bulletin of the World Health Organisation (80) 7.

Mensah, P., D. Yeboah-Manu, K. Owusu-Darko and A. Ablordey, 2002. Street foods in Accra, Ghana: How safe are they?. Bull. World Health Organ., 80: 545-555

Mossel, D.A.A., Corry, J.E.L., Struijk, C.B and Baird, R.M (1995). *Essentials of the Microbiology of Foods*. Wiley. Chichester

Nicolau, A., and Turtoi, M. (2006) Factorii care influenţeazădezvoltareamicroorganismelor, Academica, Galaţi, Pp. 264

Novasima, G.A. (2005). The meaning of water activity.Novasima. www. novassima.com.

Phimolsiripol, Y and Suppakul, P (2016). Techniques in Shelf Life Evaluation of Food Products. *Reference Module in Food Sciences*. Elsevier, pp 1- 8

Powitz , W.R. (2007). Sanitarian‟s file. Water Activity: A New Food Safety Tool.

Prescott, M.L., Harley, B.J and Klemm, A.D. (2002). Microorganisms Growth in Food.: In . Prescott, M.L., Harley, B.J and Klemm, A.D (Eds). Microbiology. Pp 964965. 5th ed. Mc Grow-Hill Companies.

Rahman, S.M. (2010) Food preservation: overview. CRC Press, Boca Raton, FL, USA, pp.3-18.

Rahman, S.M. (2010). Food stability determination by macro-micro region concept in the state diagram and by defining a critical temperature. *Journal of Food Engineering*, 99: 402-416.

Reynolds, A., Moffatt, C.R.M., Dyda, A., Hundy, R.L., Kaye, A.L., Krsteski, R., Rockliff, S., Kampen, R., Kelly, P.M and O‟Brien, E.D (2010). An outbreak of gastroenteritis due to *Salmonella* Typhimurium phage type 170 associated with consumption of a dessert containing raw egg. *Communicable diseases intelligence* 34, 329–333.

Roos, Y. H. (1993). Water activity and physical state effects on amorphous food stability. *Journal of Food Processing and Preservation* 16:433–447.

Sandulachi, E. (2012). Water Activity Concept and Its Role in Food Preservation. Technical University of Moldova.

Scott, W.J. (1953). Water relations of food spoilage microorganisms. *Advances in Food Research*, 7: 83-94

Thiruvengadam, K. V., Subramanian, N., Sharma, A. V., Krishnaswamy, S., and Nalini, S. (1973). Treatment of typhoid. *British medical journal*, *1*(5853), 612.

Wikipedia (2018). https://en.wikipedia.org/wiki/Shito Accessed on November 1, 2018

APPENDIX

Total aerobic count on Registered Shito

Parameter				
Table Analyzed	registered			
One-way analysis of variance				
P value	< 0.0001			
P value summary	***			
Are means signif. different? (P < 0.05)	Yes			
Number of groups	3			
F	281.6			
R square	0.9895			
ANOVA Table	SS	df	MS	
Treatment (between columns)	8.324e+011	2	4.162e+011	
Residual (within columns)	8.867e+009	6	1.478e+009	
Total	8.412e+011	8		

Total Aerobic count on Non-registered Shito

Parameter				

Table Analyzed	nonregistered			
One-way analysis of variance				
P value	< 0.0001			
P value summary	***			
Are means signif. different? (P < 0.05)	Yes			
Number of groups	6			
F	242.5			
R square	0.9902			
ANOVA Table	SS	df	MS	
Treatment (between columns)	1.967e+014	5	3.935e+013	
Residual (within columns)	1.947e+012	12	1.622e+011	
Total	1.987e+014	17		

Total Coliform count of registered and nonregistered shito samples

Parameter				
Table Analyzed	TCC			
One-way analysis of variance				
P value	0.0004			
P value summary	***			
Are means signif. different? (P < 0.05)	Yes			

Number of groups	4			
F	85.36			
R square	0.9846			
ANOVA Table	SS	df	MS	
Treatment (between columns)	7.810e+009	3	2.603e+009	
Residual (within columns)	1.220e+008	4	3.050e+007	
Total	7.932e+009	7		