KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI, GHANA

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Evaluation of Filamentous Mould and Aflatoxin Contamination in Processed Bottled "Shito" Sold in the Tema Metropolis

By

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DECLARATION

This research which was carried out by selecting bottled "shito" samples from about 25 supermarkets in the Tema Metropolis is my original work presented towards an award of an MSc. in Food Quality Management and that, to the best of my knowledge, it contains no material previously published by another person nor submitted to any other university or institution for the award of degree except where due acknowledgement has been made in the text. However, references from the work of others have been clearly stated.

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DEDICATION

This dissertation is dedicated to my family, for taking up some of my duties as a parent to support me as I schooled. I am very grateful, and I appreciate all they have done for me.

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My greatest appreciation and thanks goes to the almighty God for seeing me through this course and project successfully. My special thanks go to the Food Research Institute of Ghana for giving me the opportunity to have my microbiological analysis in their laboratory. Sincerest gratitude goes to my able supervisor F.C. Mills-Robertson of the Department of Biochemistry and Biotechnology, KNUST for the precious advice and invaluable time and effort he devoted to critically scrutinize my work. My deepest appreciation goes to my family for their support and encouragement throughout the project.

God richly bless each and every one.

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ABSTRACT

Foods exposed to certain filamentous fungi (moulds) could be contaminated by secondary metabolites which are mostly poisonous to humans, animals and other microorganisms. "Shito", a widely used name for hot black pepper sauce made from fish and blended spices, is ubiquitous in the Ghanaian cuisine. It may be prepared locally or commercially and consumed by all and sundry in Ghana without further processing. This study evaluated the contamination by filamentous fungi and aflatoxin in commercially processed "shito" in the Tema Metropolis. Mould was enumerated on malts extract agar by the spread plate method while identification was done by three point inoculation method on malt extract agar (containing Lactic acid), oxytetracycline-glucose yeast extract (OGYE) agar (containing OGYE supplement (SR00073), and Dichloran Rose Bengal chlortetracycline (DRBC) agar (containing chloramphenicol supplement, SR0078). A Cecil-Adept Binary Pump HPLC coupled with Shimadzu 10AxL fluorescence detector (Ex: 360nm, Em: 440) with Phenomenex HyperClone BDS C18 Column (150 x 4.60mm, 5 µm) were used for the determination of the Aflatoxins. The mould count ranged from 10^3 cfu/g to 4.4 x 10^4 cfu/g against Ghana standard Authority limit of 1.0 x 10^2 cfu/g. Genus of moulds identified were *Cladosporium*, *Aspergillus*, *Emericella*, *Rhizopus*, *Aureobasidium*, and *Penicillium* with *Penicillium* spp as the most prevalent mould in the "shito" samples, occurring in about 69% of the 30 samples analyzed. The results from aflatoxin analysis showed traces of Aflatoxin G1, and B2 in only samples 6 (S6) from Market-C. There was 0.0185 µg/g of aflatoxin G1 and 0.0314µg/g of aflatoxin B2, a total of 0.0499µg/g of aflatoxin content. Thus, regulatory bodies such as the Food and Drug Authority and Ghana standards Authority may have to conduct routine examination of "shito" because the accumulation of aflatoxin B2 over time may have the potential to cause harm,as it is a known carcinogen.

TABLE OF CONTENTS

CONTENT	PAGE
Cover Page	I
Declaration	II
Dedication	III
Acknowledgement	IV
Abstract	V
Chapter One	1
Introduction	1
Background study	1
Problem Statement	3
Justification	4
Main Objective	5
Specific Objectives	5
Chapter Two	6
2.0. Literature review	6
2.1. Food safety	6
2.2. Causes of food borne Illness	7
2.3. Food borne microorganism	7
2.3.1. Bactria	7
2.3.2. Viruses	9
2.3.3. Protozoa	10
2.3.4. Yeast	10
2.4. Mould	10
2.4.1. Morphological characteristics of Mould	12
2.4.2. Physiological characteristics of Mould	13
2.4.3. Type of Mould	14
2.4.4. Growth of Mould	15
2.4.5. Symptoms of Mould exposure	15
2.4.6. Sources of Mould and Prevention	16

2.4.7. Cancer related disease from aflatoxin produced by Moulds 16
2.5. Factors that influence Microbial growth 17
2.5.1. Extrinsic Factors 17
2.5.1.1. Temperature 17
2.5.1.2. Availability of Oxygen 18
2.5.1.3. Humidity 18
2.5.2. Intrinsic factors 18
2.5.2.1. pH of food 18
2.5.2.2. Water Activity 19
2.6. Mycotoxin 19
2.6.1. Major groups of mycotoxins 20
2.6.2. Mycotoxin Toxicity 23
2.7. Effect of Microorganism in spoilage 24
2.7.1. Chemical change 24
2.7.2. Physical changes 25
2.8. Shito 25
2.8.1. Contamination of Shito 26
Chapter Three 28
3.0. Materials and Methods 28
3.1. Study Area 28
3.2. Sample and Sample Collection Technique 29
3.3. Information on Shito 30
3.4. Chemicals, Media and reagent 31
3.5. Methods 31
3.5.1. Sterilization of Equipments and Materials 31
3.5.2 Microbial Sample Preparation 31
3.5.3 Microbial Enumeration 32
3.5.4 Identification of Moulds 32
3.6. Estimation of aflatoxin in the Shito by HPLC 32
3.7. Statistical Processing of Results 33

Chapter Four 34
4.0. Results 34
4.1. Load of Mould 34

KNUST

4.2. Identified Mould 35
4.3. Mould Isolated from each Market 36
4.4. Aflatoxin Analysis of Shito Samples 37
Chapter Five 38
5.0 Discussion 38
Chapter Six 44
6.0. Conclusions and Recommendations 44
6.1. Conclusions 44
6.2. Recommendations 44
References 45
Appendices 64

KNUST

LIST OF TABLES

Table 2.1: Statistics on cancer & liver cancer 17

Table 4.1: Load of Mould 34

Table 4.2: Mould Isolated from Shito samples from different Markets 37

Table 4: Raw Data result for enumeration analysis 64

Table 5: Anova for mould count between the shito samples 70

Table 6: Anova for mould count in the shito sample between the markets 70

Table 7: Confidence Level 77

KNUST



LIST OF FIGURES

Figure 2.1. Septate hyphae and Non Septate hyphae of Mould 13

Figure 2.2. Flow chart for shito preparation 27

Figure 3.1. Map of Tema Metropolis 29

Figure 4.1. Load of Mould in the samples 35

Figure 4.2. Percentage of individual Mould Species Isolated 36

LIST OF PLATE

Plate1. Representative plates showing various kinds of mould isolated 37

KNUST

CHAPTER ONE

1.0. INTRODUCTION

1.1. BACKGROUND INFORMATION

Food safety is a scientific discipline that describes preparation, storage, and handling of food in ways that prevent foodborne illness. Food safety has become a constant concern all over the world for leading healthcare institutions and governments of several countries to find ways to monitor production chains. It is a main concern for many people as almost everyone for the past years has ever experienced foodborne illness at least once (WHO, 2015). Most foods are prepared in open-air public spaces either itinerant or stationary, on foot or from mobile outlets, fixed outlets or removable outlets without enclosed space (Food & Agriculture Organization of the United Nations, 2016).

Foodborne Illnesses may be carried or transmitted to people through food by microorganisms (bacteria, viruses, fungi, and parasites), chemicals, or physical hazards. They usually arise from mishandling, preparation stages, or food storage stages and therefore, best sanitation and personal hygiene practices in the whole process of food or food preparations can help reduce the probabilities of contracting any form of illness.

According to the Food and Agricultural Organization (FAO) and World Health Organization (WHO), microbiological vulnerabilities are the greatest well-known risk from street foods in Africa (WHO, 2005). In Ghana, the Ministry of Food and Agriculture and World Bank, reported that a ratio of one is to forty people aches from foodborne illnesses yearly with over 420,000 reported incidents and 65,000 deaths (Ministry of Food and Agriculture/World Bank, 2007). Examples of the foodborne pathogens responsible for these foodborne illnesses include *Staphylococcus aureus, Listeria*, *Salmonella, Bacillus* and *Escherichia coli* (Foriwaa-Ababio, 2014). Likewise, Saba and Gonzalez-Zorn (2012) said that the most prevalent microscopic

organisms in Ghanaian cuisines were *Enterobacter* spp., *Citrobacter* spp., *Klebsiella* spp., and *Escherichia* spp., and stated that reviews on microbiological food security were on the decrease (http://www.who.int/foodsafety/publications/consumer/manual_keys.pdf).

The issue of food security is determined notwithstanding huge endeavours made to diminish the rate of certain sickness causing pathogens in nourishments through better homestead practices and nourishment controls. In Ghana, food laws and directions incorporate the Nourishment and Medications Law, 1992 (PNDCL 305B), Creatures (Control and Importation) Law (Top 247), Maladies of Creatures Act, 1961 (Act 83), Food and Medications (Revision) Act, 1996 (Act 523), Tourist Board Declaration 1973 (NRCD 224), Ghana Traveler Board (Alteration) Announcement, 1977 (SMCD 80) and the Nearby Government Act, 1961 (Act 54); 1993 (Act 462) (Sefa-Dedeh, 2009). The cleanliness standards at present times are not lawfully official but rather are conventions which the business should use to guarantee sustenance wellbeing (Yeleliere *et al*., 2017).

Filamentous fungal growths are metabolically adaptable living organisms with a wide existence in nature. Most are viewed as nature's essential degraders since they discharge a wide assortment of hydrolytic chemicals that degrade waste materials. Filamentous parasites such as *Aspergillus*, *Penicillium*, *Fusarium*, *Alternaria*, *Acremonium*, *Cladosporium* and *Curvularia* are regular life forms found in dirt, air and contaminated food (Gołofit-Szymczak and Górny, 2010) due to their pervasive nature and the fact that growths can develop on any substrate under ideal conditions that can be found everywhere (Klein and Paschke, 2004). Filamentous fungi are additionally known makers of optional metabolites mycotoxins which have been explored to have unfavourable wellbeing consequences for people and other creatures when presented to them (Meerdink, 2002; Muri *et al*, 2009; Wagacha *et al* 2008). Aflatoxin B1 formed by *A. flavus* and

A. parasiticus has been grouped by the Worldwide Organization for Exploration on Growth (IARC) as cancer-causing agent and as one of the aetiologies of liver disease (IARC, 2002). Chronicles of literature reviewed have shown that, the most principal bacteria isolated in Ghanaian foods are *Enterobacter* spp., *Escherichia* spp., *Staphylococcus* spp., and *Pseudomonas* spp. while the most contaminated foods were stew, soup, "fufu", macaroni, salad, and "waakye" (Yeleliere *et al*, 2017). Thus, with the growing awareness of food safety worldwide, the safety and quality of food consumed on a daily basis be continually evaluated in Ghana.

In this study ready-to-eat black pepper sauce commonly referred to as "shito" (often enjoyed with "kenkey" and other foods) was evaluated, *in vitro*, from contamination by filamentous fungi (moulds) and aflatoxins.

1.2. PROBLEM STATEMENT

Hazardous or contaminated food has been a human medical issue since history. Governments everywhere throughout the world are doing their best to enhance the security of the food supply; nonetheless, the event of foodborne ailment remains a noteworthy medical problem in both developed and developing countries. It has been estimated that every year 1.8 million individuals die due to diarrhoea with a large portion of these cases ascribed to contaminated food or water (http://www.who.int/foodsafety/publications/consumer/manual_keys.pdf).

"Shito" (ready-to-eat black pepper sauce) is either prepared for consumption in homes, for students in the boarding house or for commercial purposes. A well prepared "shito" should have a good shelf life; however improper handling and processing may expose it to contamination by moulds and consequently aflatoxin. Mould spores have been found to be part of dust in housing settings and certain commercial areas but when these mould spores are found in high amount,

they can pose a health risk to some living organisms, possibly triggering immune response plus respiratory problems (Bush *et al*., 2006). Secondary metabolites (mycotoxin) produced by certain moulds can cause devastating health risk to living organisms (Curtis *et al*., 2004). Exposure of children to mouldy foods which are likely to contain aflatoxin may lead to stunted growth and delayed development as well as liver cancer and liver damage. As it is understood that supreme security is never accomplished, numerous nations have strived to confine presentation on aflatoxin by forcing administrative breaking points on items planned for use in food. Currently, there is scanty information on the mould contaminations of "shito" in Ghana, hence, the need for such an investigation.

1.3. JUSTIFICATION

Most Ghanaian cuisines are enjoyed with "shito". Almost all and sundry consume this delicacy so it very important to consider how safe "shito" is when prepared and stored for consumption. Information from the study will reveal the presence or absence of secondary metabolites of moulds in "shito" and if possible the levels. It is also hoped that the load and types of filamentous fungi will be known.

1.4. MAIN OBJECTIVE

To evaluate filamentous mould and aflatoxin contamination of selected "shito" from supermarkets in the Tema Metropolis.

1.5. SPECIFIC OBJECTIVES

1. To determine the load of moulds in the selected "shito"
2. To microbiologically characterize the isolated moulds in the selected "shito" 3. To determine the presence and levels of aflatoxin in the selected "shito" **CHAPTER TWO**

2.0. LITERATURE REVIEW

2.1. FOOD SAFETY

Food safety is defined as the assurance that food will not cause harm, injury or sickness when it is prepared and consumed according to it intended use (Lawley *et al*., 2008). Food safety is guaranteed when levels of physical hazards, chemical hazards, and microbiological hazards are within accepted limits or maximum residual limits (Canadian Food inspection manual, 2014). In spite of the fact that legislatures everywhere throughout the world are doing their best to enhance the security of food supply, the event of foodborne sickness related with the level of nutrition remains a huge medical problem in both developed and developing nations (WHO, 2006). To guarantee safe food, taking care of, getting ready and putting away of food ought to be in an appropriate way to best reduce the danger of people getting to be wiped out with foodborne diseases (Santacruz, 2016).

2.2. CAUSES OF FOODBORNE ILLNESSES

Foodborne illness is caused by orally taking in contaminated foods or beverages. There are so many types of foodborne illnesses since different types of pathogens, which contaminate food,

cause it. Most foodborne diseases are infections caused by different types of bacteria, viruses, and parasites as well as poisonings caused by harmful toxins or chemicals that have contaminated food.

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2.3. FOODBORNE MICROORGANISMS

The microbial groups important in foods include bacteria, yeasts, protozoa, moulds, and viruses. Most bacteria, protozoa, a few moulds, and viruses cause food borne illnesses while others possibly cause food spoilage (Doyle and Buchanan, 2013).

2.3.1. Bacteria

Bacteria are unicellular organisms, which measure about 0.2µm to 1µm in diameter (Benini *et al*., 2002). Not all microscopic organisms are harmful to people, but some harmful microbes may be available in diets when they are consumed. For example, raw foods including meat, poultry, fish and shellfish, eggs, unpasteurized milk and dairy items, frequently contain microscopic organisms that influence foodborne sicknesses. Food can be contaminated during food preparation in an eatery or home, particularly if the caterers and other food handlers do not completely wash their hands, kitchen utensils, cutting sheets, and other kitchen surfaces that come into contact with raw food.

Bacteria found on food causes spoilage by digesting the food and releasing acids and other products (Doyle and Buchanan, 2013). Sometimes in the process of digesting the food, the byproducts produced can destroy the bacteria itself, affect the sensory attributes of the food and also be detrimental to human health (Jill, 2001). Most pathogenic bacteria that cause foodborne illnesses include *Salmonella* spp. *Campylobacter* jejuni, *Shigella*, *Salmonella* spp. and many others. They mostly belong to the Enterobacteriaceae family. They are Gram negative, rodshaped

facultative anaerobes that do not form spores and are mostly motile with cell width of about 0.7–1.5 µm, lengths of about 2 to 5 µm, and peritrichous flagella (Fabrega and Vila, 2013).

Salmonella spp. are Gram negative, facultative anaerobes that occur as rods. They do not form spores and are largely motile with cell diameters of about 0.7-1.5µm, lengths of about 2 to 5µm, and peritrichous flagella (Fabrega and Vila, 2013). *Salmonella* spp. tend to be ubiquitous and have been found in several animals ranging from mammals, birds, reptiles and amphibians, fish and even some invertebrates (Sting *et al*., 2013). *Salmonella* can grow within temperatures of 5.2 – 46.2 °C, pH of 3.8 – 9.5 and a water activity of 0.94 – 0.99 (FSAI, 2011). Food sources from which *Salmonella* have been isolated include eggs, pork, fish, beef, fresh fruits and vegetables, milk, infant formulas, light soup, water, salads, live and dressed chicken among many others.

Campylobacter jejuni is a Gram-negative, spiral-shaped bacterium originate in raw or undercooked chicken and unpasteurized milk. *C. jejuni* infection has been identified as the bacteria that causes acute gastroenteritis (Schlossberg, 2015) in developing countries. *Campylobacter* species are also important cause of childhood morbidity caused by diarrhoea illnesses. They are also among the most prevalent causes of diarrhoea in travellers from developed countries (Acheson and Allos, 2001).

Shigella is Gram-negative and can survive with or without Oxygen, are nonspore-forming, nonmotile, rod shaped genetically like *E. coli*. It is spread from individual to individuals and are present in the stools of people who are infected. In the event that individuals who are contaminated do not wash their hands altogether in the wake of utilizing the washroom, they can infest food that they handle or plan (Yabuuchi, 2002).

Escherichia coli is a Gram-negative, facultative anaerobic, rod shaped, coliform bacterium of the family *Escherichia* that is normally found in the lower stomach of warm-blooded living organisms (Tenaillon *et al*., 2010). It includes a few distinct strains, however just a couple of

them cause sickness in people. For example, *E. coli* O157:H7 is the strain that causes the most serious disease.

Listeria monocytogenes is a small rod-shaped (0.5-2 μm x 0.5 μm), non-spore forming Grampositive bacterium which is isolated in small chains and motile at 20-25 °C. It is classified as a part of the genus *Listeria* along with *L. ivanovii, L. innocua, L. welshimeri, L. selligeri* and *L. grayi* (Rocourt *et al*., 2007). *L. monocytogenes* has been found in raw and undercooked food.

Clostridium botulinum is a Gram-positive, anaerobic, spore-shaping, motile bacterium with the capability to deliver the neurotoxin botulinum (Lindström *et al*., 2006; Peck, 2009). The botulinum poison can cause an extreme flaccid immobile illness in people and different creatures (Lindström *et al*., 2006) and is a strong poison known to mankind, regular or engineered, with a toxic measurement of 1.3– 2.1 ng/kg in people. *C. botulinum* may be found in preserved food, salted fish. Meats, unpasteurized milk, soft cheeses, and ready-to-eat deli meats and hot dogs.

2.3.2. Viruses

A virus is a microscopic organism that replicates inside living cells of diverse creatures. Viruses may contaminate a wide range of living things, from human beings and plants to microorganisms and archaea (Koonin *et al*., 2006). Viruses found in food are most of the times infected by personnel or live organism with the virus (carrier) which the food comes in contact with (Prescott, 2002). Food handlers with viral infections who do not take any precaution to prevent the transfer of the sputum, sweat, breath unto a food material are likely to be the carriers of food borne illnesses caused by viruses. If personnel do not wash and sanitize their hands after using the washroom, this becomes an ideal mode of transfer of viruses unto food immediately the person eats. The two most important viruses of concern in food-borne outbreaks are hepatitis A and Norwalk-like viruses (single-stranded RNA viruses) (Schwab *et al*, 2000**)**.

2.3.3. Protozoa

They are eukaryotic, unicellular living beings. Motion is a characteristic related with many species and characterized by how they move (some utilize flagella, others utilize cilia, and others utilize pseudopodia) with some other species being non-motile. They mostly exist in an endless assortment of shapes since they have no cell walls. Numerous species cause such human diseases as loose bowels (Doyle and Buchanan, 2013).

2.3.4. Yeast

Yeasts are eukaryotic, single-celled microorganisms that can develop in products with a low pH (5.0 or lower) and present, natural acids, and other effortlessly used carbon sources (Kurtzman, 2006). Yeasts can be in charge of the disintegration of nourishment with high sugar content. A similar impact is helpful in the creation of different kinds of sustenance and drinks, for example, bread, yogurt, juice, and mixed refreshments (Jill, 2001)

2.4. MOULDS

Moulds are non-motile, filamentous, branched fungi that use spores for reproduction. The cell wall is composed of cellulose, chitin, or both and composed of hyphae which may be asexual or sexual. The sexual hypha usually spreads in the air and forms exospores, either free (conidia) or in a sack (sporangium).

The term mould is applied to multicellular, filamentous fungi whose growth on food is usually readily recognized by its fuzzy or cottony appearance (Lasztity *et al*., 2009). Generally, moulds are concerned with the spoilage of foods and mouldy or mildewed food is considered unfit for consumption. Moulds can be used in the manufacture of different foods and are ingredients of

some foods. For instance, some kinds of cheese such as Roquefort and Camembert are mouldripened. They may be grown as feed and food and may also be employed to produce products used in foods, such as amylases and other enzymes for bread making or citric acid used in soft drinks (Ghori *et al*., 2009). Moulds are major contributors in the ripening of many oriental foods. A species of *Bothrytis cinerea* is responsible for the noble rot of grape. Moulds are also used for the production of several antibiotics. For instance, some members of the genus *Penicilium* produce penicillin, a molecule that is used as an antibiotic, which kills or stops the growth of certain kinds of bacteria inside the body.

Penicilium expansum is one of the most prevalent post-harvest rots that infects apples. Although it is a major economic problem in apples, this plant pathogen can be isolated from a wide host range, including pears, strawberries, tomatoes, corn, and rice. Mould also produces the carcinogenic metabolite patulin, a neurotoxin that is harmful in apple juice and apple products. The levels of patulin in food products are a health concern because many are consumed by young children. In addition, a second secondary metabolite citrinin is produced as well.

Penicillium viridicatum is a psychrophilic species of the genus of *Penicilium* which produces xanthomegin, xanthoviridicatin D, xanthoviridicatin G, viommellin, viridamine viridicatumtoxin, pectinase, penicillic acid and citrinin and can spoil grapes and melons. Mycotoxin production by the various species of *Penicillium* is quite distinctive and may be used as a valuable aid in their identification.

2.4.1. Morphological Characteristics of Mould

Macroscopically, moulds comprise of a mass of spreading, interlaced fibers called hyphae (solitary hypha), and the entire mass of these hyphae is known as a mycelium. Hyphae might be classed as vegetative or fertile based on their biological functions. The vegetative hyphae or growing hyphae are concerned with the nutrition of the mould and the fertile ones with the

production of reproductive parts. The hyphae of some moulds are full and smooth, but the hyphae of others are characteristically thin and ragged.

A couple of moulds release sclerotia (singular sclerotium) which are firmly packed masses of hyphae, regularly thick-walled, inside the mycelium. These sclerotia are extensively more impervious to warm and other unfavorable conditions than whatever is left of the mycelium and thus might be critical in some prepared nourishment items. In the group of moulds called septate, the hyphae are divided by cross-walls into cells. The hyphae of the nonseptate group comprise clearly of cylinders without cross walls (Figure1).

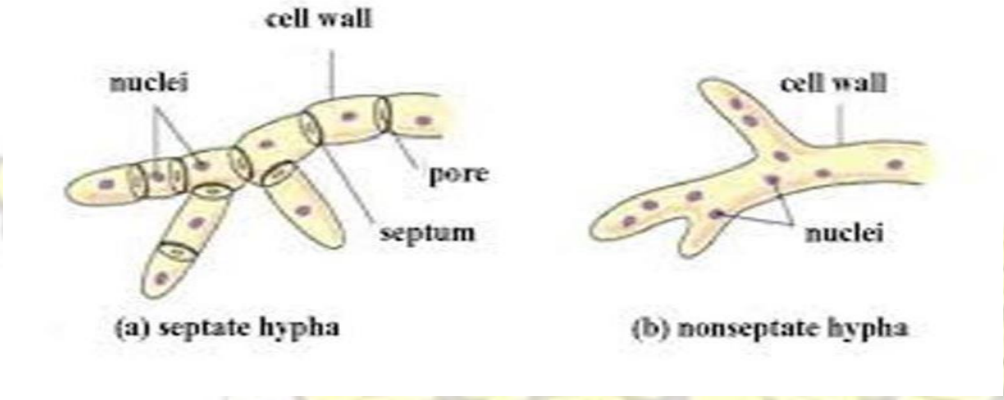


Figure 1: (a) Septate hyphae and (b) Non-septate hyphae of a Mould

The reproductive parts or structures of moulds are the spores, which are basically asexual and are produced in large numbers and readily spread via air. Spores that settle on favourable substrates can start another period of growth phase and develop into another mycelium (Pekhtasheva *et al*., 2011).

2.4.2. Physiological Characteristics of Moulds

In general, moulds require less dampness than bacteria and yeasts. Moulds vary considerably among themselves in reference to optimum water activity and range of water activity for germination of spores (Hamad, 2012). The minimum water activity for spore germination has

been observed to be as low as 0.62 for some and as high as 0.93 for others (Lasztity *et al*., 2009). Each mould has an optimum water activity and range of water activity for growth. The reduction of water activity below the optimum for a mould delays the germination of the spores and reduce the growth rate. Most moulds grow well at ordinary room temperatures and are classified as mesophilic. The optimum temperature for most moulds is between 25 and 30°C.

In any case, it should be noted that some moulds grow fairly well at temperatures of freezing or just above, and others can grow slowly at sub-zero temperatures. Moulds require free oxygen for growth and this is why moulds grow on the surface of contaminated food and most grow over a wide range of pH, but some are favoured by acid foods such as found in majority of fruits. Moulds are found in virtually every environment and can be detected, indoors and outdoors, all year round with growth encouraged by warm and humid conditions (Heseltine *et al*., 2009). Outdoors, they can be found in shady, damp areas or places where leaves or other vegetation are decomposing while indoors they can be found where humidity levels are high.

2.4.3. Types of Moulds

Some forms of mould regularly seen on meat and poultry are *Alternaria, Aspergillus, Botrytis, Cladosporium, Fusarium, Geotrichum, Monilia, Manoscus, Mortierella, Mucor, Neurospora, Oidium, Oosproa, Penicillium, Rhizopus* and *Thamnidium*. These moulds can likewise be found on numerous different foods (Weidemaier *et al*., 2013).

Aspergillus is one of the economically important moulds normally seen in warm, extremely humid climates, and a public occupant of household dust. Some *Aspergillus* species cause genuine ailment in people and creatures. For instance, *A. fumigatus* and A. *flavus* produce

aflatoxin which is both a poison and a cancer-causing agent. The most well-known species causing hypersensitive illness are *A. fumigatus* and A. *clavatus*. *Aspergillus* can cause neonatal infections.

A. fumigatus infections are primary pulmonary infections and can potentially become a rapidly necrotizing pneumonia with a potential to disseminate. The organism can be differentiated from other common mould infections based on the fact that it takes on a mould form both in the environment and in the host.

Trichothecium roseum is a fungus in the division Ascomycota. *Trichothecium roseum* produces a wide variety of secondary metabolites including mycotoxins, such as roseotoxins and trichothecenes, which can infect and spoil a variety of fruit crops (Batt *et al.,* 2014). It can act as both a secondary and as opportunistic pathogen by causing pink rot on various fruits and vegetables and thus has an economic impact on the farming industry (Batt *et al*., 2014). Secondary metabolites of *T. roseum*, specifically trichothecinol A, are being investigated as potential anti-metastatic drugs. Several agents including harpin, silicon oxide, and sodium silicate are potential inhibitors of *T. roseum* growth on fruit crops (Yang *et al*., 2005). *Trichothecium roseum* is mainly a plant pathogen and has yet to show a significant impact on human health.

2.4.4. Growth of Mould

Mould spores lie dormant on almost all surfaces. When they land on a surface with the necessary environment, however, they will germinate and produce more spores. Food is a perfect breeding ground for moulds as it is inherently high in nutrients. Even fresh food can hosts mould spores but they do not become visible until the food is a little older and the mould spores have had a

chance to germinate. Once they germinate, mould will start to appear and at this point, the food is so full of mould spores that it is no longer safe to eat (Tournas, 2005).

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2.4.5. Symptoms of Mould Exposure

Mould exposures have a variety of health effects depending on the individual. A few people are more affected by mould than others. Exposure to mould can cause various medical problems, for example, throat aggravation, nasal stuffiness, eye irritation, cough and wheezing, and sometimes skin irritation. Individuals at higher risk for mould hypersensitivities are people with chronic lung illnesses, which will result in more severe reactions when exposed to mould (Gureje et al,. 1998). Persistent pain and well-being: a World Health Organization study in primary care. (Gureje *et al*., 1998). A serious health threat from mould exposure for immunocompromised individuals is systemic fungal infection (systemic mycosis). Immunocompromised individuals exposed to high levels of mould, or individuals with chronic exposure may become infected. Sinuses and digestive tract infections are most common; lung and skin infections are also possible. Opportunistic infection by moulds such as *Penicillium marneffei* and *Aspergillus fumigatus* is a common cause of illness and death among immunocompromised people, including people with AIDS or asthma.

2.4.6. Sources of Mould and Prevention

Prevention of mould exposure and its ensuing health issues begin with prevention of mould growth in the first place by avoiding a mould-supporting environment such as humid air (Barbeau *et al*., 2010). Certain moulds excrete volatile gases in lower concentrations which may not even be detected by sensitive analytical sampling methods. The concentrations of these gases

are only determined by their smell known as ergonomic odour and they do not indicate toxicological significant exposure.

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2.4.7. Cancer Related Disease from Aflatoxin Produced by Moulds

Table 1 shows the trends of cancer prevalent in one of the big hospitals in Ghana. One of the metabolites of moulds is aflatoxin which causes cancer. The generation of cancerous cells in the body might be due to the consumption of food contaminated with aflatoxin. Various types of moulds such as *Aspergillus Flavus, A. fumigatum, Penicilium viridicatum, Emericella nidulans , Penicilum hirsutum, Penicilium expansum, Rhizoctina, Cladosporium, Penicilium italicum* can be found in "shito" . The levels of these mould in food has a significant influence on aflatoxin production which has great effects of causing cancerous cells in the body.

Table 1: Statistics on Cancer and Liver Cancer

YEAR	CANCER	LIVER CANCER	PERCENTAGE (%) OF LIVER CANCER
2012	728	6	0.82
2013	743	19	2.56
2014	769	5	0.65
2015	746	10	1.34
2016	786	21	2.67

(Source: Biostat Department, Komfo Anokye Teaching Hospital, 2017).

2.5. FACTORS THAT INFLUENCE MICROBIAL GROWTH

Microbial growth in food are affected by two major factors which are extrinsic and intrinsic factors.

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2.5.1. Extrinsic Factors

These are factors which are outside the food (storage conditions of the food) but affect the growth of the microorganism. They include Temperature, Humidity and available oxygen.

2.5.1.1. Temperature

Almost every kind of microorganisms has a defined temperature range in which they mature, with a minimum, maximum, and optimum temperature. Temperatures have dramatic impact on both the generation time of an organism and its lag period (Valero and Serrano, 2015). Mesophiles are microorganisms that live and thrive at moderate temperatures. Psychrophiles thrive at temperatures much lower than normal while thermophiles thrive at comparatively high temperatures. A small number of microorganisms are able to survive at temperatures close to 0 ^{0}C and below 5 ^{0}C where growth of most spoilage organisms are retarded while most pathogens stops (Prescott *et al*., 2002).

2.5.1.2. Availability of Oxygen

Aerobic bacteria are microorganisms which are able to survive in the presence of oxygen while anaerobic bacteria survive without oxygen (Jay, 2002). *Pseudomonas* species are examples of aerobic bacteria while *Clostridium* species are examples of anaerobes (Adam and Moss, 2008). Facultative anaerobes can utilize oxygen; however, they can metabolize without oxygen. Microaerophiles require minimal oxygen while aerotolerant organisms are functionally

anaerobic, as they do not have a terminal O_2 acceptor, but can function in the presence of oxygen (Miller-Keane *et al*., 2003).

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2.5.1.3. Humidity

Every living organism requires water for survival just like microbes. Food with high moisture content is susceptible to microbial growth. Microorganism are grouped based on the water survival level, Bacteria are able to thrive at water level of 92% followed by yeast which thrives at 90% and Moulds at 85-90% (Mariott *et al*., 2006).

2.5.2. Intrinsic Factors

The food in which the microbe grows creates a medium which promote the proliferation of the microbes. Intrinsic factors include the pH of the food, the Nutrient and Water Activity.

2.5.2.1. pH of Food

Each microorganism has a maximal and an ideal pH for development. In general, yeasts and moulds are more acidic tolerant than bacteria. The inherent pH of food varies although most are neutral or acidic. Food with low pH value (below 4.5) usually are not readily spoiled by bacteria but are susceptible to spoilage by yeasts and moulds (Mbwiga, 2016). Most microorganisms thrive at neutral medium, pH of 7.0 (Jay, 2002).

2.5.2.2. Water Activity

Microorganisms have absolute demands for water, without which no growth can occur. The exact amount of moisture required for development of microorganisms differs. The moisture requirement is expressed in terms of water activities. Reducing the amount of water present in

the food alters the proliferation of microbial activities in the food (Adam and Moss, 2008). The water activity refers to water available for metabolic activities; the optimum water activity is about 0.99 for most microbes. A water activity of 0.91 is good for bacteria growth.

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2.6. MYCOTOXINS

A mycotoxin is a toxic secondary metabolite produced by moulds and is capable of causing disease and death in both humans and animals. Certain moulds of the same species can produce several kinds of mycotoxins, and various species may release similar mycotoxin (Youssuf Gherbawy *et al*., 2010). Mycotoxins normally get into the food chain when food eaten is contaminated by mould or livestock consume such infected food which are then consumed by living organisms including humans.

In 2004 in Kenya, 125 people died and nearly 200 others were treated for aflatoxicosis after eating aflatoxin-contaminated maize. The deaths were mainly associated with home-grown maize that had not been treated with fungicides or properly dried before storage. This was attributed to famine, where food producers were harvesting maize earlier than the due time for harvesting to prevent thieves from stealing their crops from the field. Thus, there were grains which had not completely developed therefore making it highly susceptible to infection. Spices are susceptible substrate for growth of mycotoxigenic fungi and mycotoxin production. Red chilli, black pepper, and dry ginger were found to be the most contaminated spice (Norhayati *et al*., 2015).

2.6.1. Major Groups of Mycotoxins

Aflatoxins are a kind of mycotoxin produced by *Aspergillus* species such as *A. flavus* and *A. parasiticus*. The umbrella term aflatoxin refers to four different types of mycotoxin produced,

which are B1, B2, G1, and G2. Aflatoxin B1, the most toxic, is a potent carcinogen and has been directly correlated to adverse health effects, such as liver cancer, in many animal species (Martin *et al*., 2001). Aflatoxins are largely associated with commodities produced in the tropics and subtropics, such as cotton, peanuts, spices, pistachios, and maize.

Ochratoxin is a mycotoxin consisting of three secondary metabolite forms, A, B, and C produced by *Penicillium* and *Aspergillus* species. Differences exist among the three forms in that Ochratoxin B (OTB) is a non-chlorinated form of Ochratoxin A (OTA) and that Ochratoxin C (OTC) is an ethyl ester form of Ochratoxin A. *Aspergillus ochraceus* is seen as a contaminant of an extensive variety of products involving beverages such as beer and wine. *Aspergillus carbonarius* is the primary species seen in vine juice, which discharges its poison during the juice making process.

Citrinin is a toxin that was initially isolated from *Penicillium citrinum* however it has been recognized in over a dozen species of *Penicillium* and several species of *Aspergillus*. Some of these species are utilized to produce human food stuffs such as cheese (*Penicillium camemberti*), sake, miso, and soy sauce (*Aspergillus oryzae*). Citrinin is associated with yellowed rice desease in Japan and acts as a nephrotoxin in every single creature specie tested. In spite of the fact that it is associated with numerous human foods (wheat, rice, corn, barley, oats, rye, and food coloured with Monascus pigment) (Saito *et al*., 1971), its full significance for human wellbeing is unknown. Citrinin can likewise act synergistically with Ochratoxin A to depress RNA synthesis in murine kidneys (Doughari, 2015).

Ergot alkaloids are compounds produced as a toxic mixture of alkaloids in the sclerotia of species of *Claviceps*, which are normal pathogens of various grass species. Ingestion of ergot sclerotia from contaminated cereals, commonly in the form of bread produced from contaminated flour, cause ergotism, the human illness generally known as St. Anthony's fire.

There are two main kinds of ergotism: gangrenous, influencing blood supply to extremities, and convulsive that influences the central nervous system (Craig *et al*., 2015). In the twenty first century, certain strategies for grain cleaning have significantly reduced ergotism as a human disease; nonetheless, it is as yet a critical veterinary issue (Craig *et al*., 2015).

Patulin is a toxin produced by the *P. expansum, Aspergillus* and *Paecilomyces* species. *P. expansum* is particularly associated with a scope of mouldy fruit and vegetables, specifically, decaying apples and figs. It is destroyed by the fermentation process and therefore not found in apple drinks, such as cider. Although patulin has not been shown to be carcinogenic, it has been reported to damage the immune system in animals (Moses, 2008). In 2004, the European Community set limits to the concentrations of patulin in food products, currently, at 50 µg/kg in all fruit juice concentrations, at 25 µg/kg in solid apple products used for direct consumption and at 10 µg/kg for children's apple products, including apple juice (Lubinda *et al*., 2014) **Fusarium** toxins are produced by more than 50 species of *Fusarium* and have a history of contaminating the grain of developing cereals such as wheat and maize. They include a range of mycotoxins such as fumonisins, which affect the nervous systems of horses and may cause cancer in rodents; the trichothecenes, which are most strongly associated with chronic and fatal toxic effects in animals or humans; and zearalenone, which is not associated to any lethal impacts in animals and humans. Some of the other major types of Fusarium toxins include:

beauvercin and enniatins, butenolide, equisetin, and fusarins (Desjardins *et al*., 2007)

Viridicatumtoxin A (viridicatumtoxin) is a fungus-derived tetracycline-like antibiotic found in *Penicillium viridicatum* and *Penicillium aethiopicum*, among other fungi (Chooi *et al*., 2010). Viridicatumtoxin A inhibits growth of *Staphylococcus aureus*, including methicillin resistant *S.*

aureus and quinolone-resistant *S. aureus*, with an activity 8 to 64 times greater than that of tetracycline (Zheng *et al*., 2008).

Viridicatumtoxin B is a fungus-derived tetracycline-like antibiotic isolated from small number of *Penicillium* fungi. A synthetic structure matching that of natural viridicatumtoxin B makes possible synthetic variants that match or surpass its antibiotic potency. Analogs lacking a hydroxyl group were even more effective than the original against Gram-positive bacteria (Zheng *et al*., 2008).

Although various wild mushrooms contain an assortment of poisons that are definitely fungal metabolites causing noteworthy health problems for humans, they are rather arbitrarily excluded from discussions of mycotoxicology. In such cases, the distinction is based on the size of the producing fungus and human intention. Mycotoxin exposure is almost always accidental whereas with mushrooms, improper identification and ingestion causing mushroom poisoning is a common case. Ingestion of misidentified mushrooms containing mycotoxins may result in hallucinations. The cyclopeptide-producing Amanita phalloides is well known for its toxic potential and is responsible for approximately 90% of all mushroom fatalities. The other primary mycotoxin groups found in mushrooms include: orellanine, monomethylhydrazine, disulfiramlike, hallucinogenic indoles, muscarinic, isoxazole, and gastrointestinal (GI)-specific irritants (Karimi *et al*., 2013).

2.6.2. Mycotoxin Toxicity

Environmental conditions affect the production of mycotoxins at the transcription level. Temperature, water movement and pH, strongly impact mycotoxin biosynthesis by increasing the level of transcription inside the fungal spore. It has likewise been discovered that low levels of fungicides can help mycotoxin synthesis. Certain mycotoxin can be harmful or lethal to

humans and animals when exposure is high enough (Hussein and Brasel, 2001). Extreme exposure to very high levels of mycotoxin can lead to neurological problems and in some cases death; fortunately, such exposures rarely or never occur in normal exposure scenarios, even in residences with serious mould problems. Prolonged exposure, such as daily workplace exposure, can be particularly harmful.

It is thought that all moulds may produce mycotoxin and consequently all moulds may be potentially toxic if sufficiently large amounts are ingested, or humans become exposed to extreme quantities of the mould. Mycotoxin is not produced constantly, but only under specific growing conditions. Mycotoxin can be found on the spores of moulds and mould fragments, and in this manner, they can likewise be found on the substrate upon which the mould grows. Routes of entry for these moulds can include ingestion, dermal exposure and inhalation. Some mycotoxins cause immune system responses that vary considerably, depending on the individual. The duration of exposure, the frequency of exposure and the concentration of the mould are elements in triggering immune system response. Aflatoxin is an example of a mycotoxin, a cancer-causing poison produced by certain fungi in or on foods and feeds, especially in field corn and peanuts (MESSRIPOUR and Gheisari, 2010).

2.7. Effect of Microorganisms on Spoilage

Microorganisms in food cause detrimental changes in colour, flavour, odour and texture (Davidson, 2003) and this makes the food not fit for consumption. The changes can be either chemical or physical changes.

2.7.1. Chemical changes

Certain microorganisms are able to cause chemical changes in food through the production of enzymes such as lipases. The lipases break down fat and this leads to rancidity resulting from microbial activities. Other microbes hydrolyze complex molecules into simpler compounds which serve as a source of nutrient for their survival. The preferred food substance for microbial activity is carbohydrate and the effect of the microbial activities results in alcohol and organic acid production which alter the chemical state of the food (Jay, 2002).

2.7.2. Physical Changes

There are more physical changes in food by microorganism than chemical changes (Doyle, 2007). Some food items may become softer than normal. If the microorganisms are moulds, they often become visible externally on the food (Dugan *et al*., 1997). Such visible moulds depend on free oxygen to grow in food to cause food spoilage. Other moulds grow inside the food and either depend on oxygen or no oxygen for spoilage (Herden, 2000).

2.8. "SHITO"

Shito is the name mostly used for hot black pepper sauce commonly used as part of Ghanaian dishes (Lawley *et al*., 2002). "Shito" sauce is primarily made of fish, vegetable oil, ginger, garlic, dried fish, prawns, crustaceans, tomato paste, dried powdered peppers and spices among others. Figure 2.1 shows is a flow chart of how "shito" is prepared. The blend of spices and fish differs between different regions and villages but owes its original recipe to the Ga tribe. "Shito" is not always hot black pepper and it can also be prepared without the use of oil. The ingredients for this type of "Shito" are fresh pepper, onions, tomatoes and a little salt mashed together in an earthenware bowl popularly known as "asanka" and a pestle. The colour of the resulting sauce is

red ("shitor tsulu") or green ("kpakpo shito") depending on the colour of the pepper used (Mensah *et al*., 2002).

In Ghana, "shito" is served with varieties of dishes and it can be taken in with "banku", "akple", "gari", "kenkey" and boiled rice (Mensah *et al*., 2002). Indeed, its use is similar to ketchup, hot sauce or chili oil (Antwi-Agyei and Maalekuu, 2011). It is scarce to find "Shito" served with white bread or spring roll. In some Chinese restaurants in Ghana, "Shito" is used in the stead of hot oil as complement dish to fried rice, plain rice and others (Anim-Antwi, 2011). It is not uncommon to find "shito" being eaten with white bread or spring rolls. In most Chinese restaurants across Ghana, "shito" replaces hot oil as a condiment to fried rice or plain rice (Anim-Antwi, 2011). The nutrition aspect of "shito" is that it contains mainly ground fish and vegetables which are sources of proteins and vitamins in addition to fat and oil, respectively.

2.8.1. Contamination of "Shito"

Whenever food is handled personally by human beings, there is always the possibility of addition of human pathogens (Newell *et al*., 2010). In a study carried out by Yeboah Manu *et al*. (2010), there was a consideration of contaminated food both in the open markets and in restaurants on the University of Ghana campus. It was found out that most of the food had high levels of contaminations. The target of their study was to find Aerobic Colony Count (ACC), total Enterobacteriaceae (EC), presence of *Escherichia coli* and the presence of *Salmonella* ssp. as well *Shigella* spp. All these contaminants were found in both open market food and the restaurants. The authors, therefore, called for more severe measures by the public health department of the university and Ghana as a whole to help address the situation. "Shito" as an open market or street vended food served at most restaurants in Ghana is prone to contamination.

In a study carried out by Saba and Gonzalez-Zion (2012), "shito" samples were found to contain *Klebsiella cloacae, K. pneumonia, E. cloacae,* and *E. coli.* These organisms have serious health implications. For instance, *K. pneumoniae* normally found in healthcare settings causes bloodstream infection, urinary tract infections as well as pneumonia (Saba and Gonzalez-Zion 2012). *E. coli* is a type of microorganism that regularly live in the digestive organs of individuals and creatures. A few kinds of *E. coli*, especially *E. coli* O157:H7, can cause intestinal disease. *E. coli* O157:H7 and different strains that leads to intestinal disorder are called Shiga toxinproducing *E. coli* (STEC) (Schulman *et al*., 2017).

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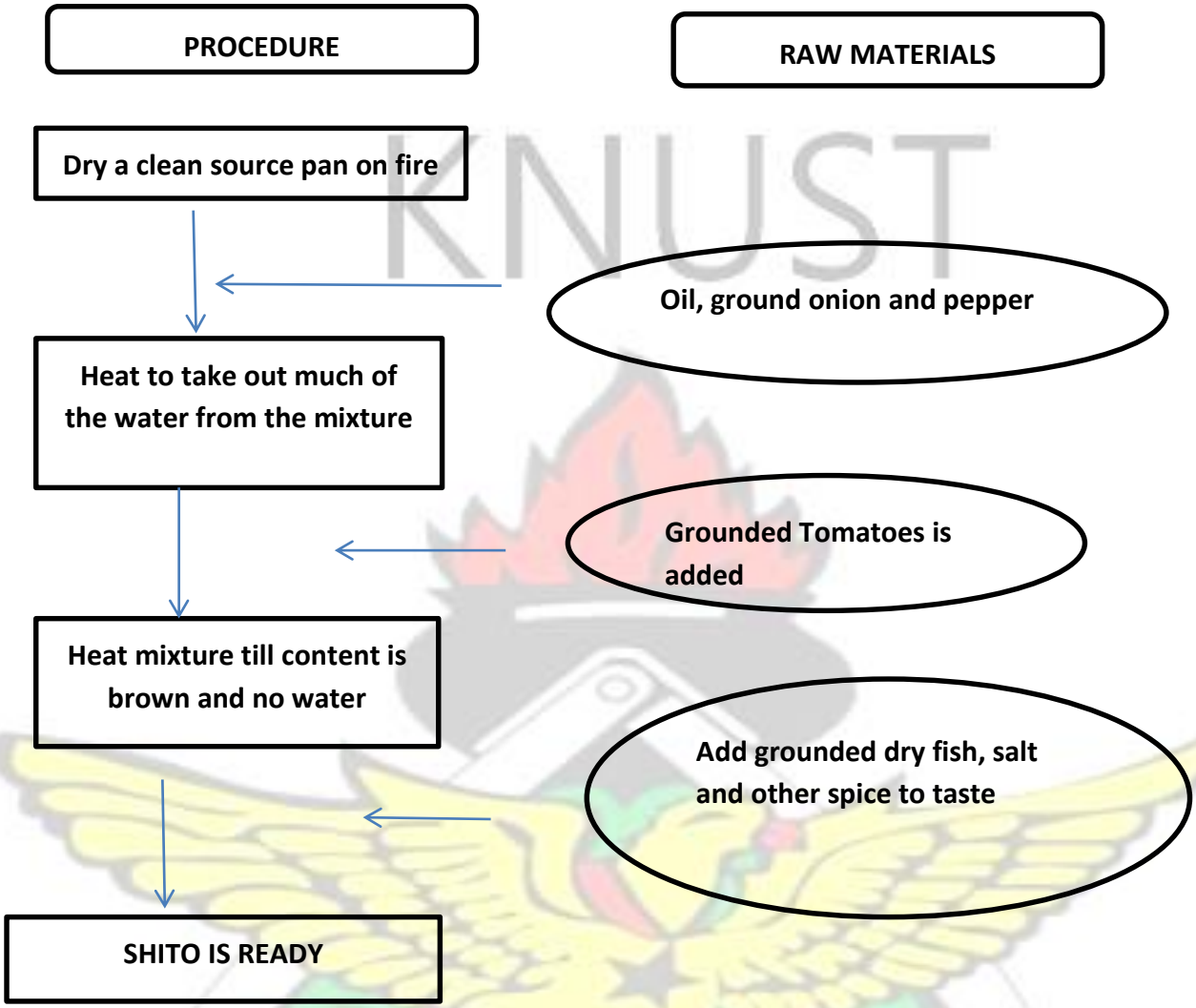


Figure 2.1. Flow Chart for "Shito" Preparation CHAPTER THREE

3.0. MATERIALS AND METHODS

3.1. STUDY AREA

Tema Metropolitan area is a coastal district situated about 30 kilometers east of Accra, the capital city of Ghana. It shares boundaries on the North East with the Dangme West District (DWD), Southwest by Ledzekuku Krowor Municipal, Northwest by Adenta Municipal and the Ga East Municipal (GEMA) North by the Akuapim South District and the south by the Gulf of Guinea. The Ashaiman Municipal is in-lock district within the Tema Metropolitan Assembly.

The Metropolis covers an area of 396 km and lies within the coastal savannah zone. The Greenwich meridian (Longitude 0) passes through the Metropolis, which meets the equator or latitude 0 in the Ghanaian waters in the Gulf of Guinea. The southern tip of the Metropolis lies on the latitude 541 north. The Metropolis proximity to the sea with its low-lying terrain which projects into the sea makes a natural endowment for harbour hence the construction of Tema harbour making the district ―The Eastern Gateway of Ghana (GIS, 2014).

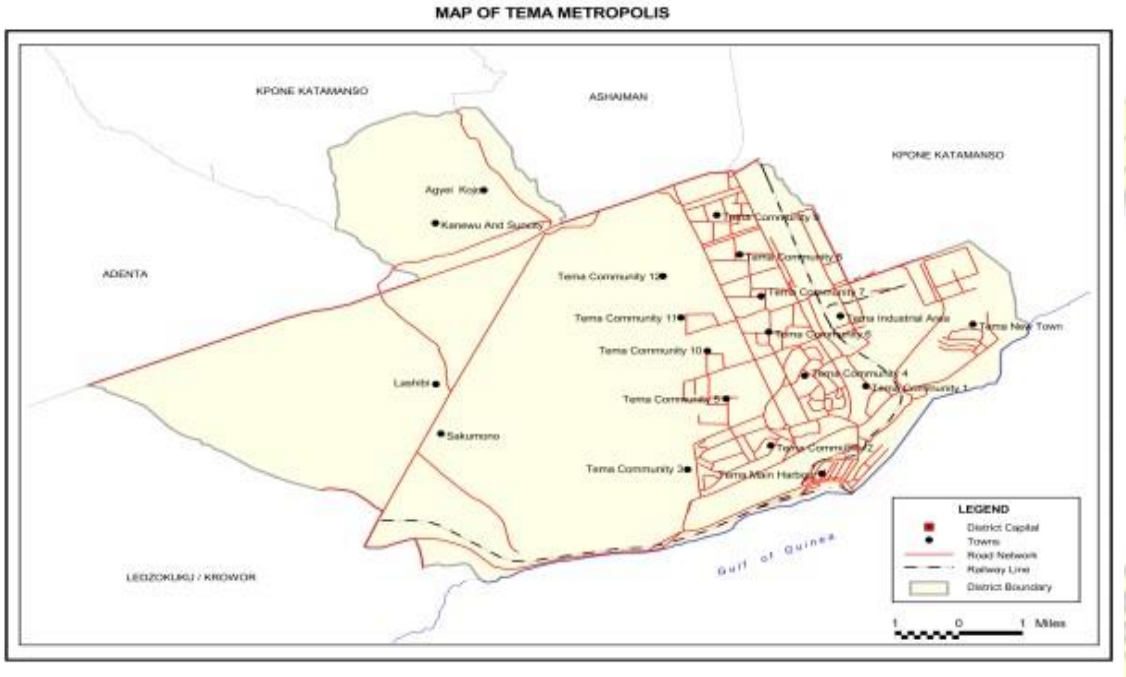


Figure 3.1. Map of the Tema Metropolis

(Source: GIS, 2014)

3.2. Sample and Sample Collection Technique

The collection of the samples took place from October 2016 to December 2016 at five markets in the Tema Metropolis. The five markets were coded as Market A, Market B, Market C, Market D and Market E. "Shito" samples considered for the study were bought packaged from different vendors. Samples were collected in sterile bottles and analyzed within 24 h of sampling from the markets and those that were not analyzed the same day were stored in the refrigerator at 4°C. A sample was prepared in the laboratory using the basic recipe, to serve as a control sample by the use of the procedure stated in Figure 2.1.

3.3. INFORMATION ON "SHITO" SAMPLE

	SHITO SAMPLES FROM TEMA METROPOLIS			
MARKETS	NAME OF SAMPLE	PRODUCTION DATE	EXPIRY DATE	REGISTRATION STATUS
	MV FDS PEPER SAURCE	Jun-15	Jun-17	GSA
	JOECARL	Aug-17	Feb-18	GSA/FDA AD 03-013
	CYNLAND		Dec-16	FDB/5514-015
	ESMA	Oct-16	Oct-17	GSA 1800
	CHRISCOP	Jun-16	Jan-18	FDA/SS 10-002
A	NANA ADEBI		Apr-17	FDA/SS 14-008
	MUMY'S DELICIOUS		Oct-17	
	AUNTY ESE'S		Aug-17	
	DAVIS SPECIAL SHITO	Sep-16	Sep-17	FDAVM1312
	OHEMAA WOYE JE SHITO	Sep-16	Aug-18	FDA/SS 15-007
	STUDENT COMPANION	Jul-17	Mar-18	FDA 15-005
B	MELODY SPECIAL	Jun-16	Jun-17	

	ANGELA	Sep-15	Dec-18	
	ESISEL	Oct-16	Oct-17	
	KESSMAY		Apr-17	
	MONIBAS	Oct-16	Oct-17	FDA/SS 15-02
	GOOD'S		Oct-17	GSA
C	OSPAT CATERING SERVICES		May-16	
	JIJI		Oct-17	GSA, FDB/AD 06-00
	UNIQUE FOOD COMPANION		Feb-17	
	ORCHIDS SHITO	Feb-16	Jan-17	
	HOME FRESH FOOD	Feb-16	Feb-19	
	LORD PROVIDE SPECIAL SHITO	Jun-16	Jun-17	FDA/SS 15-039
D	MAX MART		Feb-17	GSA, FDA/AD06-004
	HAJIA MUNI	Nov-15	May-17	GSA
	GRACE SHITO		Oct-17	
	ABRAHAM DELICIOUS	Apr-16	Oct-17	GSA STD.N.GS240:2001
	ERMAKS		Dec-16	
E	NEW GENERATION		May-18	

3.4. CHEMICALS, MEDIA AND REAGENTS

Bacteriological peptone water (Oxoid LP0037), Dichloran Rose Bengal Chlortetracycline (DRBC) agar (Fluka analytical 17147), Oxytetracycline Glucose Yeast Extract (OGY) agar (Oxoid CM0545), and Malt Extract agar (Wade road, Basingstoke, Hants).

3.5. METHODS

3.5.1. Sterilization of Equipment and Materials

Materials used for this study were subjected to sterilization under laboratory conditions using standard procedures (Cheesbrough, 2009). Test tubes and glass petri dishes were washed

thoroughly with soap and water, rinsed, air dried and sterilized in an oven at 170 °C for two hours. In order to prevent the media from getting contaminated, all media and inocula were prepared under aseptic condition within the fume chamber with the ultra violet light turned on for about 30 minute before use. Furthermore, open ends of all test tubes containing media and samples were sterilized using naked flame before and after inoculum transfer. The inoculation needles were flamed before and after each inoculation. Culture media were also only opened when they were ready to be used and the work benches thoroughly cleaned with 70% isopropanol before and after work.

3.5.2. Microbial Sample Preparation

Working under aseptic condition, ten grams (10 g) of each sample was weighed using a sterile weighing bag which contained 90 mL sterile peptone water (Egbuta *et al*., 2014). Each sample was vortexed for about 1 min at moderate speed and serially diluted to make four dilutions (10^{-1} - 10^{-4}) by transferring 1 mL homogenized sample to 9 mL dilution blank, mixing well until the 10^{-4} dilution was obtained. Aliquots (0.1 mL) of these dilutions were used for the study.

3.5.3 Microbial Enumeration

Spread plate method of inoculation was employed in the microbial examination of the samples. From the prepared 10-fold serial dilutions, enumeration of moulds was carried out by the spread plate method on malt extract agar. Bacteria growth on the media was inhibited by the addition of 10% lactic acid. The petri dishes were incubated at 25 °C for 5 to 7 days. After the incubation periods, the plates were selected and manually counted. The number of colony-forming units per

gram (cfu/g) of samples was calculated by multiplying the number of organisms by the dilution factor.

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3.5.4. Identification of Moulds

Four different media; malt extract agar (MEA) (containing Lactic acid), oxytetracycline-glucose yeast extract (OGYE) agar (containing OGYE supplement (SR00073), and Dichloran Rose Bengal chlortetracycline (DRBC) agar (containing chloramphenicol supplement, SR0078) were used for the identification of the moulds. Each type of morphological colony found on the malt extract agar were picked from each plate with the aid of a sterile inoculation needle, and subcultured onto the three types of media using the three-point inoculation methods. The plates were then incubated at 25 °C for 5 to 7 days.

3.6. ESTIMATION OF AFLATOXIN IN THE SHITO BY HPLC

A Cecil-Capable Paired Pump HPLC combined with Shimadzu 10AxL fluorescence finder (Ex: 360 nm, Em: 440) with Phenomenex HyperClone BDS C18 Segment (150 x 4.60 mm, 5 µm were utilized. The versatile stage utilized was methanol:water (40:60, v/v) at a stream rate of 1 mL/min with section temperature kept up at 40°C. To 1L of portable stage was added to 119 mg of potassium bromide and 350 µl of 4M nitric corrosive (required for post section electrochemical derivatisation with Kobra Cell, R-Biopharm Rhone). Aflatoxin Blend (G1, G2, B1, B2) gauges (ng/g) were set up from Supelco aflatoxin standard of 2.6 ng/µL in methanol. Grouping of B1 and G1 were 0.5, 1, 2, 8, 16 ng/g per 100 µl infusion. Convergence of B2 and G2 were 0.15, 0.3, 0.6, 2.4, 4.8 ng/g per 100 µl infusion. Breaking point of Location and Farthest point of Evaluation for add up to aflatoxin were built up at 0.5 ng/g and 1 ng/g individually (Akowuah *et al*., 2015)

3.7. STATISTICAL PROCESSING OF RESULTS

Analysis was done in triplicates, to minimize the error margin as much as possible. Results obtained were tabulated using Microsoft Excel 2010 for easy interpretation. The data was subjected to one-way analysis of variance (ANOVA) and the significant differences between the means of the various markets determined by using Microsoft Excel (version 2016). P-values ≥ 0.05 were considered as statistically not significant.

CHAPTER FOUR

4.0. RESULTS

4.1. LOAD OF MOULDS

All the counts from the enumeration analysis were subjected to logarithm to the base of ten. The results from each of the markets are shown in Table 4.1. Market-A recorded highest value of log 4.3 ± 0.13 cfu/g in MS2 and a lowest log value of log 3.3 ± 0.3 cfu/g in MS4. For Market-B there was no count in all of the six samples (MS1-MS6). For Market-C, the highest value was log 3.4 ± 0.22 cfu/g for samples from MS6 and a lowest value of log 1.0 ± 1.73 cfu/g in MS4. With Market-D, the highest recorded was log 3.52 ± 0.07 cfu/g in MS5 with the lowest being log 1.05 ± 1.82 cfu/g in sample from MS3. Market-E recorded log 3.76 ± 0.20 cfu/g in samples from MS5

and a lowest of log2.36 ± 2.04 cfu/g in samples from MS6. The control samples had no mould growth.

Table 4.1: Load of Mould

Samples	MARKETS /log10 cfu/g					
	Market A	Market B	Market C	Market D	Market E	Control
MS1	3.40±0.17	NIL	NIL	NIL	NIL	NIL
MS2	4.31± 0.13	NIL	2.1± 1.73	NIL	2.36± 2.04	NIL
MS3	4.26 ± 0.35	NIL	2.1 ± 1.8	1.05 ± 1.82	NIL	NIL
MS4	3.3 ± 0.3	NIL	1.00 ± 1.73	NIL	3.49± 0.20	NIL
MS5	3.84± 0.57	NIL	NIL	3.52 ± 0.07	3.49 ± 0.20	NIL
MS6	NIL	NIL	3.4 × ±0.22	3.16 ± 0.28	3.76± 0.15	NIL

Key: CT – control, NIL – No mould count, MS1 – market sample '1', MS-2 – market sample '2', MS-3 – market sample '3', MS-4 – market sample '4', MS-5 – market sample '5', MS-6 – market sample '6'

4.2. IDENTIFIED MOULDS

Moulds belonging to six genera were isolated and identified from all the "shito" samples. The different genera included *Cladosporium, Aspergillus, Emericella, Rhizopus, Aureobasidium,* and *Penicillium* (Table 4.2). Figure 4.1 shows the different organisms isolated from the various "shito" samples from the different markets. Four different species of *Aspergillus,* twenty of *Penicillium* species, one *Cladosporidium* species, two *Emericella* specie, one *Aurobasidium* and one *Rhizopus* species were identified in "shito" samples bought from the various markets. *Penicillium viridicatum* was found to be the most prevalent mould species in the shito samples, occurring in sixteen of 30 samples representing 55%. *A. flavus* occurred in three of thirty samples (10%), *Emericella nidulans* and *P.expansum* occurred in two of the thirty samples respectively

(7%), with *P.hirsutum*, *P.italicum*, *A.fumigatus*, *Rhizoctonia solani*, *Cladosporium cladosporoides* and *Aurobasidium pollulans* occurring once out of the thirty samples (3%) (Figure 4).

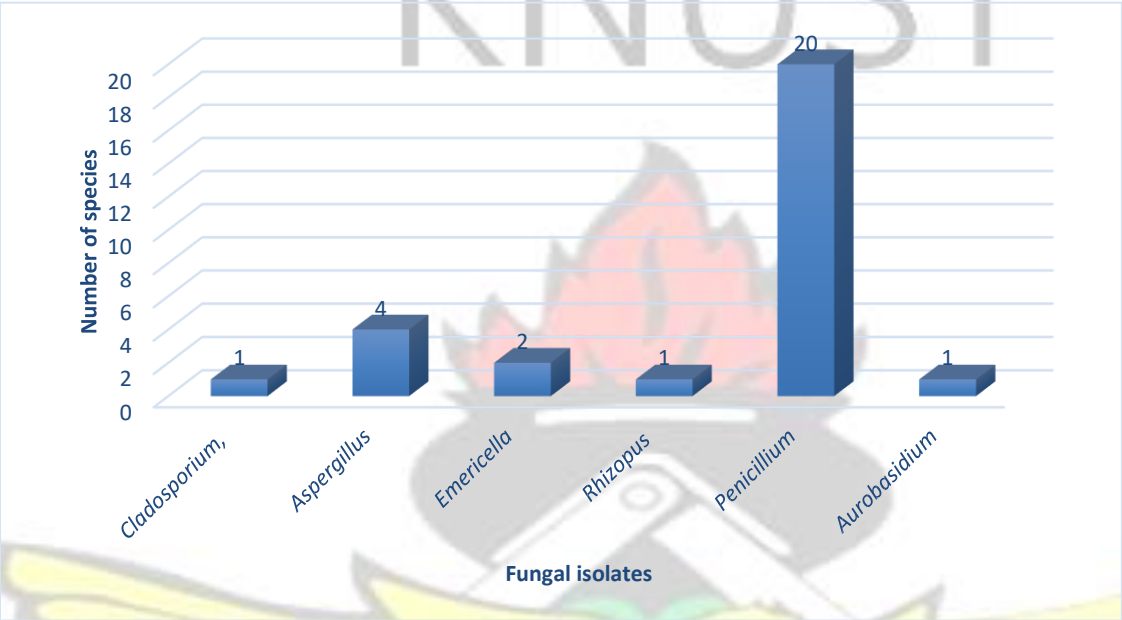


Figure 4.1:Mould Load and types in Shito Samples

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NYANSAPO WO SANE NO BADWENMA

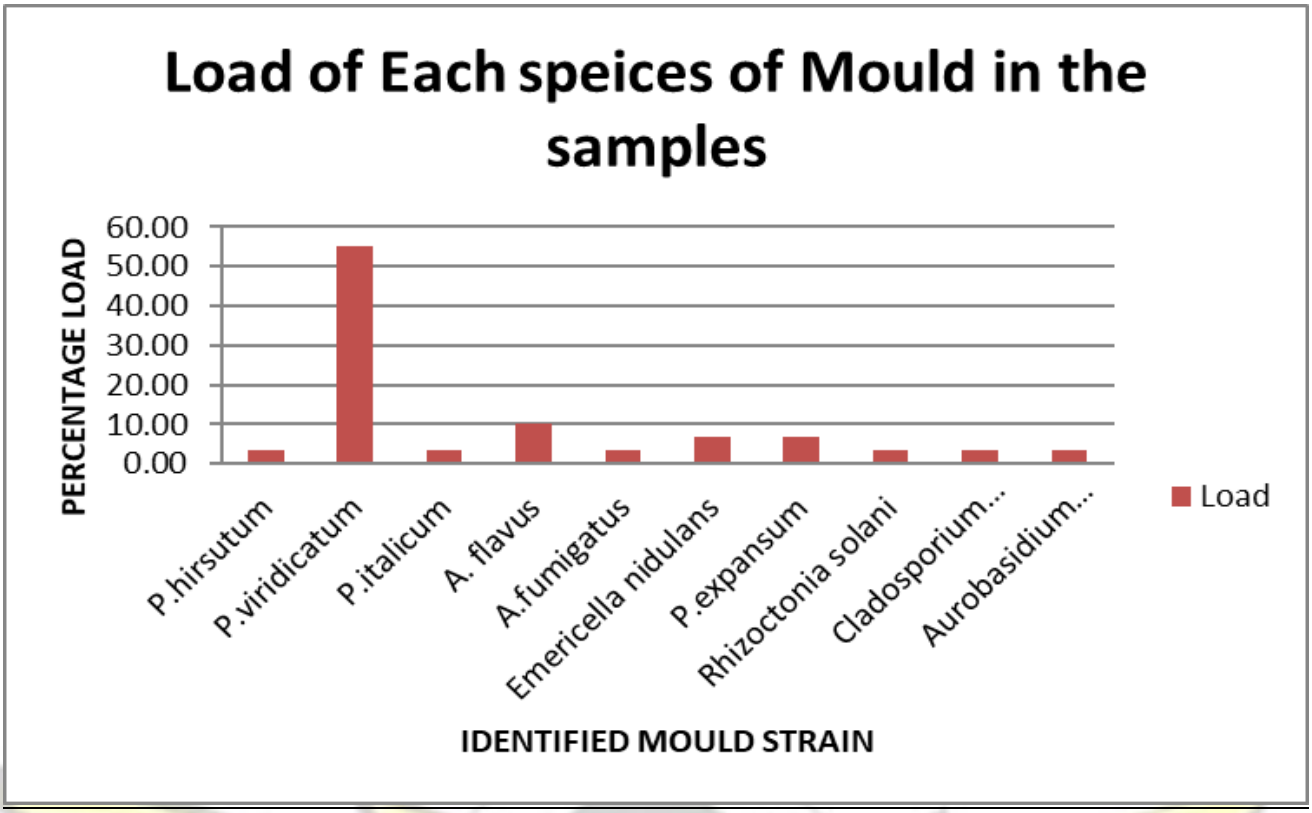


Figure 4.2.: Percentage of individual Mould species isolated

4.3. MOULD ISOLATES FROM EACH MARKET

Apart from the Market-2 vendors and the Control that did not have any mould contamination, samples from the other four markets had mould contaminants. *Penicillium* species were identified in all the samples and were also the most predominant species identified in all the Markets, except Market B (Table 4.2). The second most common identified species was *Aspergillus* Species, identified in Market-A and Market-C. Market-C had about five different genuses of mould species, making it the Market that revealed different varieties of fungal contaminants. Plate 1 shows some of the moulds identified.

Table 4.1: Moulds Isolated from 'shito' Samples from the different Markets

SHITO SAMPLES FROM MARKET	ORGANISM ISOLATED IN THAT MARKET
MARKET-A	*Penicillium hirsutum, Penicillium viridicatum, Aspergillus flavus, Emericella nidulans*
MARKET-B	*None*
MARKET-C	*Penicillium viridicatum, Penicillium expansum, Rhizoctonia solani,Cladosporium cladosporoides, Aspergillus fumigatus, Penicillium italicum, Aurobasidium pollulans*
MARKET-D	*Penicillium viridicatum*
MARKET-E	*Penicillium viridicatum*

4.4. AFLATOXIN ANALYSIS OF THE SHITO SAMPLES

The results from aflatoxin analysis showed traces of Aflatoxin G1and B2 in only market sample 6 (MS-6) from Market-C at a level of 0.0185 µg/g of aflatoxin G1 and 0.0314 µg/g of aflatoxin B2, with a total of 0.0499 µg/g of aflatoxin content realized.

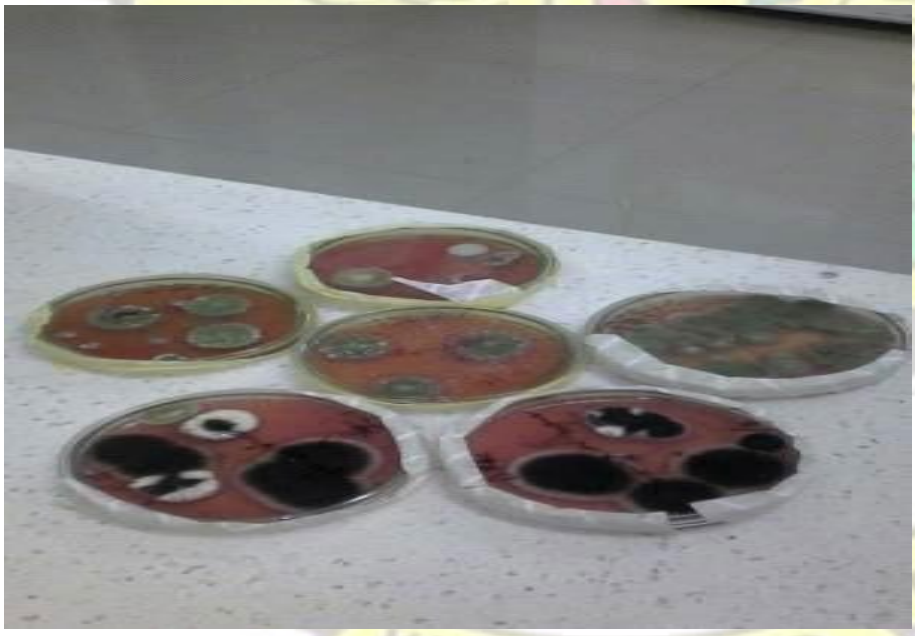


Plate 1: Representative plate showing various kinds of moulds isolated

CHAPTER FIVE

5.0. DISCUSSION

"Shito" processed for commercial purposes or for sale in supermarkets are required to be processed under very hygienic conditions which are certified by any of the regulatory bodies, such as the Food and Drugs Authority (FDA) and Ghana Standards Authority (GSA). These "shito" products should be free from moulds if it is well prepared and stored at the appropriate conditions. In this study, thirty samples were analyzed and fifteen were found to be contaminated by moulds with nine out of these fifteen registered with either FDA or GSA. Six were not registered by any of the regulatory authorities.

In this study, Market-A had five samples contaminated out of the six samples with counts higher than the 100 cfu/g set by the Ghana Standard Authority (GSA: 240:2001). This may reflect the level of hygiene and good manufacturing practices awareness level of the Vendors in Market-A. For Market-B, there was no contaminant in any of the six samples analysed. This could mean that the vendors followed the highest hygienic and good manufacturing practices. They might have ensured that the equipment used for the "shito" preparation were adequately clean and free from producing any form of contamination and under clean environment devoid of any form of contamination.

Market-C had four of the six samples contaminated with counts higher than the standard set by Ghana standard Authority. For Market-D, two samples recorded contaminations with mould counts higher than the limit set by the Ghana Standard Authority (Table 4.1). Aside the threshold (100 cfu/g) set by Ghana standard Authority, all the contaminations were beyond the threshold of 1000 cfu/g set for mould counts in foods by the Woolworths Quality Assurance (2012).

Several scientific studies on street food in Ghana have detected high levels of microbial food contamination. A study by Mensah *et al*. (2002) in Accra, found that the microbial quality

sampled street foods such as salads, macaroni, “fufu”, “omo tuo” and red pepper had unacceptable levels of contamination. Mesophilic bacteria were detected in 69.7% of foods. *Bacillus cereus* was detected in 5.5% of the foods, 31.9% contained *S. aureus* and 33.7% contained Enterobacteriaceae. Also, *Shigella sonnei* and enteroaggregative *E. coli* were isolated from macaroni, rice, and tomato stew with *Salmonella arizonae* isolated from light soup. A recent study by Atter *et al*. (2015), on safety of street vended traditional maize beverage, and ice-kenkey, in Ghana, revealed the presence of coliform bacteria, *E. coli*, *S. aureus*, aerobic mesophiles, yeasts and moulds in Accra and Tema. This trend of microbial contaminations is common to most street foods including processed bottled “shito”.

The “shito” samples in this study had about 69% of *Penicillium* contaminants, 14% *Aspergillus*, 7% *Emericella*, 4% *Rhizoctina*, 3% *Cladosporuim,* and 3% *Aurobasidium* (Figure 4.2). This compares well with work by William and Bialkowska (1993). The *Penicillium* species included *P. viridicantum, P. expansum*, *P. hirsutum* and *P. italicum* with the most predominant strain of being *P. viridicatum* followed by *P. hirsatum. Penicillium* is an extensive and universal family which as of now contains 354 acknowledged species (Visagie *et al*., 2014). Numerous species are basic contaminants on different substrates and are known as potential mycotoxin producers (Lyratzopoulos *et al*., 2002)

Penicillium are of major importance in the natural environment as well as food and drug production. Some members of the genus produce *penicillin*, a molecule that is used as an antibiotic, which kills or stops the growth of certain kinds of bacteria while other species are used in cheese making (Kirk *et al*., 2008). Notwithstanding their significance in the food business, some species of *Penicillium* and *Aspergillus* serve in the generation of various biotechnologically created chemicals and different macromolecules, for example, gluconic, citric and tartaric acids, and in addition a few pectinases, lipase, amylases, cellulases, and proteases.

Some *Penicillium* species have indicated potential for use in bioremediation, due to their capacity to separate an assortment of xenobiotic mixes (Leitao, 2009).

Some *Aspergillus* species cause candid illness in living organisms with the most renowned pathogenic species being *A. fumigatus* and *A. flavus*, which produce aflatoxin that is both a poison and a cancer-causing agent. Some species of *Aspergillus* such as *A. niger* is a major source of citric acid gobally which is used in flavouring and preservation of soft drinks and beverages (Newell *et al*, 2010).

Other moulds isolated were *Emericella nidulans, Rhizoctonia solani*, *Cladosporium, P. italicum,* and *Aurobasidium pollutant*. *Cladosporium* species are not known to produce mycotoxins and are rarely pathogenic to humans; however, studies have shown that spores produced by these organisms may produce allergic reaction in asthmatic patients and people with respiratory problems (Hasnain *et al*., 2004).

Personal hygiene of personnel preparing the "shito" is of key importance in the prevention of contamination of "shito" by microbes. Parts of the body that contribute to contamination of food include the skin, hand, hair, eyes, mouth, nose, nasopharynx, respiratory tract and excretory organs. These parts of the body act as carriers of dangerous microorganisms and cause contamination (Mariott and Gravani, 2006).

Every human being has some microbial load on his or her body, thus, it is very prudent for personnel involved in food preparation or in "shito" processing to observe good personal hygiene as well as observing good manufacturing practices. These good manufacturing practices include washing of hands well with soap before touching any cooking items, wearing clean clothes, ensuring that personnel have well-trimmed and short figure nails without polish and consistent disinfections of hands. Personnel must wear clean uniforms with hair covered and with no items

that can fall from their body, such materials as in earring, wristband and chains. Persons with ill health do not have to come into contact with the food during its preparation (Foskett and Cesaran, 2007). The frequent washing of hands during food preparation has been found to be the most effective way of controlling microbial food contamination.

The stages involved in "shito" preparation is such that, the environment plays a major role in keeping the "shito" safe for consumption. For instance, "shito" is prepared under continuous stirring of the sauce, and thus very much exposed to the environment. Air is used to serve a good purpose of cooling the "shito" after bottling or packaging since accumulation of heat on top of the packaged bottle can lead to crushing of the bottle if it is covered with accumulation of heat (Personal observation). Therefore, all forms of microorganisms in the air can get into contact with the exposed "shito" being cooled prior to packaging. Thus, it is advisable to prepare the "shito" in an enclosed clean room with treated air, which will prevent air or any other form of contamination.

The cooking temperature for "shito" is very crucial; if it is done at a high temperature it would burn and develop undesirable flavour and taste and if it is done at low temperature it is likely to have a high water activity which would increase microbial growth (Adams and Moss, 2008). Therefore, moderate temperature which would ensure low water activity and development of good flavour and taste is advised to be used. "Shito" is normally stored at room temperature for short term storage. For long term storage and to prevent spoilage due to its nutritional content it needs to be kept at a low temperature of close to 4^0C or Freezing temperature.

The Ghana standard on "shito" did not state the limit of aflatoxin in "shito" but according to the European commission, certain ready to eat food should have limits not exceeding 4ug/kg (EU,

2009). Also, the acceptable critical limit of aflatoxin in "shito" from the spices used in the preparation of the "shito" should not be more than 10ug/kg (Venâncio and Paterson, 2007). Aflatoxin B1 produced by *A. flavus* and *A. parasiticus* has been classified by the International Agency for Research on Cancer (IARC) as a Group 1 cancer-causing agent and has been reported as one of the aetiologies of liver cancer (IARC, 2002). Physicochemically, mycotoxins are thermostable and in most cases aromatic and nonantigenic low-molecular metabolites and exert a diverse range of toxic effects because their chemical structures are very heterogeneous (Doolotkeldieva, 2010).

From the analysis of variance (appendix 2) applied on the means, there was a significant difference ($P \leq 0.05$) in the mould counts among the "shito" samples. This may be attributed to the different conditions and environments for the preparation of the "shito" from the different vendors. The growth of mould prior to and after drying of the constituents of the "shito" may results in mycotoxin production, particularly Aflatoxins in spices including pepper, the main ingredient of "shito" (ICMSF, 2011). Therefore, proper drying, storage and preparation conditions to achieve a water activity below 0.6 are needed to prevent mycotoxin production (Muggeridge, 2001).

In this study, traces of Aflatoxin B2 and G1 were found in one sample of the "shito", probably, as a result of the growth of moulds and the availability of suitable moisture and temperature for fungal growth. Thus, presence of aflatoxins may depend on the type of the fungus and environmental conditions, as well as the availability of nutrients to the fungus

Pepper, a major ingredient in the preparation of "shito" is very vulnerable to aflatoxin contamination, which is affected by atmospheric temperature, humidity, insects,drying and processing conditions. Sun drying is a common postharvest practice in some countries, especially

Ghana, where peppers are spread on soil in a single layer leading to some pepper being contaminated with fungus (Iqbal *et al*., 2011; Erdogan, 2004). Though the toxicity is not high, it will be very safe if mouldy samples are not consumed because accumulation of aflatoxin B2 over time may have the potential to cause harm.

One common mould found during this study was *Penicillium expansum* that produces Patulin which has an effect on the immune system of animals. Ochratoxin is produced by most *Penicillium* species, one of the predominant strains isolated in this study. Ochratoxin produced is carcinogenic and has nephrotoxic (kidney) effect and can also cause tumour in the human urinary tract (Peraica *et al*., 2001).

It has been established that certain moulds are of great importance to the food industry as they are used in the manufacturing of certain food products like cheese, soy sauce among many others. For example, *Penicilium Viridicatum* which formed about 60% of the mould species isolated and identified in this study, produces the viridicatum toxin, a fungi-derived tetracyclinelike antibiotic that inhibit the growth of *staphylococcus aureus*, methicillin resistant *Staphylococcus aureus* (Zheng, 2008).

CHAPTER SIX

6.0. CONCLUSIONS AND RECOMMENDATIONS

6.1. CONCLUSIONS

From this research, moulds were observed in both the certified “shito” and non-certified”shito” samples. The counts observed were all above the Ghana Standard Authority limit of 10^2 cfu/g. *Penicillium* species, and *A. fumigatus* and *A. flavus* identified are known to produce aflatoxin

which is known both as a toxin and a carcinogen. Only traces of aflatoxins were observed in one sample among the 30 samples analyzed.

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6.2. RECOMMENDATIONS

Further study is proposed to focus on “shito” used before and after sales by street vendors.

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APPENDIX ONE

RAW DATA RESULTS FOR ENUMERATION ANALYSIS

MARKETS	SAMPLES NO.	FDA/GSA NO.	MEAN	SD
A	1	GSA	3.401373	0.1738
	2	GSA/FDA/AD03-013	4.309891	0.1281
	3	FDA/SS14-015	4.257929	0.3518
	4	GSA	3.30103	0.3010
	5	FDA/SS10-002	3.836848	0.5674

	6	FDA/SS14-008	0	0.0000
B	1		0	0.0000
	2		0	0.0000
	3	FDA1312-681	0	0.0000
	4	FDA/SS15-007	0	0.0000

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	5	FDA15-005	0	0.0000
	6		0	0.0000
C	1		0	0.0000
	2		2	1.7321
	3		0	0.0000

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	4	FDA/SS15-02	0	0.0000
	5	GSA	0	0.0000
	6	GSA/FDA/AD06-00	3.433677	0.2298
D	1		0	0.0000
	2		0	0.0000

KNUST

	3		0	0.0000
	4	FDA/SS15-039	0	0.0000
	5	GSA/FDAA2-15	3.518768	0.0721
	6	GSA/FDA/AD 06-004	3.15904	0.2755
E	1	GSA	0	0.0000

KNUST

	2		2.359727	2.0445
	3		0	0.0000
	4	GSA	3.492374	0.1994
	5		3.492374	0.1994
	6		3.7611	0.1512

KNUST

KNUST

APPENDIX 2

ANOVA for mould count among the 'shito' samples

	Sum of Squares	*df*	*Mean Square*	*F*	*P-value*	*F crit*
Between Groups	498.670	4	124.667	5.323	0.0030	2.758
Within Groups	585.468	25	23.418			
Total	1084.14	29				

ANOVA for mould count in the 'shito' samples between the markets

	Sum of Squares	*Df*	*Mean Square*	*F*	*Pvalue*	*F crit*
Between Groups	104.384	5	20.876	0.511	0.764	2.620
Within Groups	979.755	24	40.823			

Total		1084.139547	29

APPENDIX 3

PREPARATION OF MALT EXTRACT AGAR.

The agar was prepared by first dissolving 50g of dehydrated straw coloured free flowing powder in one litre of distilled water. The media was boiled to dissolve with continuous agitation. It then autoclaved at 115 degrees for 10min. The prepared media was dispensing into 250ml sterilized bottles in quantities of 200ml.Cooled stored at a temperature of 2-8 degrees Celsius.
Before use, the media is heated to liquid and then cooled to 55 degrees Celsius. The pH of the media is adjusted to 3.5 by adding 10% of lactic acid SR0021 to 100ml of the media.

PREPARATION OF DICHLORAN ROSE BENGAL CHLORAMPHENICOL AGAR

This media was prepared by suspending 15.75g of dehydrated pink coloured free flowing powder in 500 ml of distilled water. It was heated to complete dissolution with constant agitation. A chloramphenicol supplement vial SR0078 was reconstituted and the content of the vial added to the media. The media was sterilized by autoclaving it at 121 degrees Celsius for 15min. it was cooled to 50 degrees. The pink gel media was then poured into petri dish for it to solidify.

PREPARATION OF OXYTETRACYLINE GLUCOSE YEAST EXTRACT AGAR

18.5g of straw coloured free flowing dehydrated powder was dissolved in 500ml of distilled water and was boiled with consistent agitation for complete dissolution of media. The media was then cooled and later autoclaved at 115 degrees Celsius for 10 min. A vial of Oxytetracycline GYE selective supplement (SR0073) was reconstituted and aseptically added unto the cooled

media; 15ml of the mixture was dispensed into sterilized petri dish and allowed to solidify for use.

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IDENTIFICATION OF MOULD COLONIES.

The Colonies were theoretically identified by their physical appearance under the microscope. Penicilium is distinguished by its frequently greenish colonies and its branching or simple conidiophores supporting phialides in brush-like clusters .Macroscopically, penicilium appears in texture velvety to powdery; Green, blue-green, gray-green, white, yellow, or pinkish on the surface. Most of the colonies from the Shito plate had colonies of this description. *Aspergillus species* were greenish with white boarders and a black centre.

HPLC Determination

A Cecil-Adept Binary Pump HPLC coupled with Shimadzu 10AxL fluorescence detector (Ex: 360nm, Em: 440) with Phenomenex HyperClone BDS C18 Column (150 x 4.60mm, 5um). The mobile phase used was methanol: water (40:60, v/v) at a flow rate of 1ml/min with column temperature maintained at 40°C. To 1 liter of mobile phase were added 119 mg of potassium bromide and 350ul of 4M nitric acid (required for post column electrochemical derivatisation with Kobra Cell, R-Biopharm Rhone). Aflatoxin Mix (G1, G2, B1, B2) standards (ng/g) were prepared from Supelco® aflatoxin standard of 2.6ng/µL in methanol. Concentration of B1 and G1 were 0.5, 1, 2, 8, 16 ng/g per 100ul injection. Concentration of B2 and G2 were 0.15, 0.3, 0.6, 2.4, 4.8 ng/g per 100ul injection. Limit of Detection and Limit of Quantification for total aflatoxin were established at 0.5ng/g and 1ng/g respectively

SAMPLE PREPARATION FOR AFLATOXIN ANALYSIS

Extraction and Clean-up

A mixture of homogenized shito sample (25g) with 5g of sodium chloride and 125ml of methanol/ deionized water (70:30) was blended at high speed for 2mins and filtered through a fluted filter paper. The extract (15ml) was diluted with 30ml of deionized water and filtered through a1.0 µm microfiber filter. The diluted extract (15ml) was passed through Vicam Aflatest immunoaffinity WB column (IAC), which was washed twice with 10ml deionized water. Aflatoxin was eluted from the IAC with 1ml HPLC grade methanol and 100ul of the eluent was injected into the HPLC.

APPENDIX 4

Aflatoxin Calculation

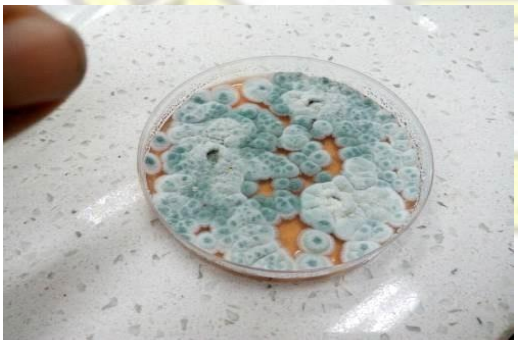
Aflatoxin, ng/g = A x (T/I) x (1/W)

Where A = ng of aflatoxin as eluate injected, T = final test solution eluate volume (ul),

I = volume eluate injected into LC (ul), W = mass (g) of commodity represented by final extract.

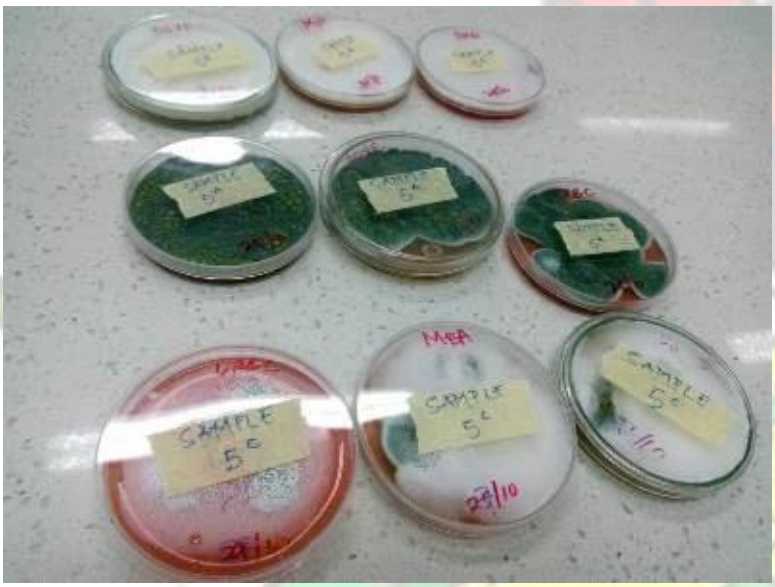
NOTE: T = 2000, I = 100, W = 1

APPENDIX 5

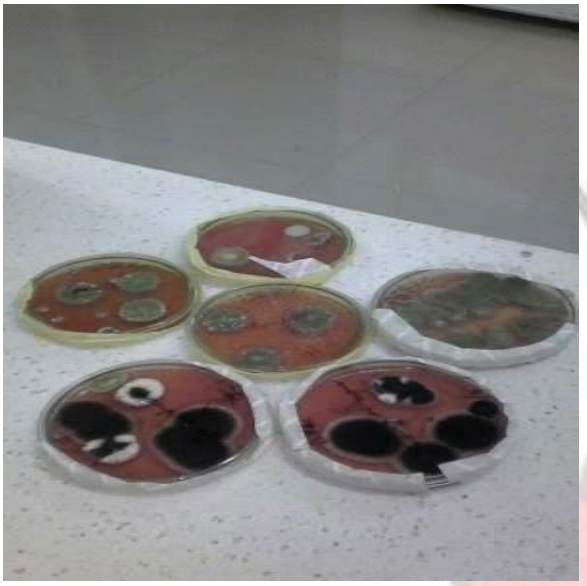




COLONIES FROM SPREAD PLATE



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SANE NO BADWENMA

COLONIES FROM IDENTIFICATION PLATES APPENDIX 6







SHITO SAMPLES

APPENDIX 7

COLLECTION OF SAMPLES

Choosing a confidence level of 90%, per the formula and critical value from the table below:

$N= \{(Z\alpha/2/2E)^{2}\}$

Where N= number of sample

E=Margin of error

Zα=Critical Value

Table 7 CONFIDENCE LEVEL

Confidence Level (1-α)g 100%	Significance E	Critical Value Zα
90%	0.10	1.645
95%	0.05	1.960
98%	0.02	2.326
99%	0.01	2.576

Using this confidence level, about 16 samples were analyzed. In order to make the sample space more economical and viable, 30 samples were taken from the shell shops of Tema metropolis.

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