ISOLATION AND CHARACTERIZATION OF FUNGI FROM FRIED BEAN CAKE (AKARA) SOLD ACROSS BENIN METROPOLIS

BY

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DEPARTMENT OF MICROBIOLOGY

FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN,

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NOVEMBER, 2022

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CERTIFICATION

This is to certify that this project work was carried out by **Sade Hajarat MAJOYEGBE** with the matriculation number LS1705575 n the Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City.

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MR. E. I OBAZENU **Date**

(Project Supervisor)

APPROVAL

This project work was carried out by **Sade Hajarat MAJOYEGBE** under the supervision of MR. E. I. OBAZENU in partial fulfillment of the award of Bachelor of Science (B.Sc) degree in the department of Microbiology, University of Benin, Benin City.

_____ _____

Prof. (Mrs) F.I. Akinnibosun **Date**

(Head of Department)

DEDICATION

This project work is dedicated to God Almighty who has been my source of strength and for his guidance throughout my life.

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My immense gratitude goes to God Almighty for his protection, wisdom, blessing and grace which saw me through the completion of this project and guidance throughout my stay in this great citadel of learning.

I also wish to express my profound gratitude to my supervisor Mr E. I. Obazenu for his support and time dedicated in making this work a success.

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ABSTRACT

This study was aimed at determining fungal contaminants in Fried bean cake sold in four Local Government Area across Benin metropolis. A total of twelve (12) samples were analyzed using standard microbiological procedures. The fungal population was isolated and identified based on its cultural and morphological characteristics. The fungi isolated were *Penicillium chrysogenum, Mucor mucedo, Aspergillus flavus, Saccharomyces* sp. and *Fusarium oxysporium*. The mean fungal count ranged from 3.7×10^4cfu/g to 1.3×10^4 cfu/g and the prevalence of these fungal isolates ranged from 33.33% to 8.33%. Though the sample does not exceed the mean microbial count recommended standard of 10^5 cfu/g for ready-to-eat foods and the total fungal count of 10^4 cfu/g for food. There might have been hygienic problems either in the processing, handling and distribution of the food. It is therefore encouraged that proper hygienic procedures are followed in the processing and handling of food to prevent the spread of microorganisms and its toxins that are of public health importance.

CHAPTER ONE

1.0 Background of study

Food is any substance consumed for nutritional support for the body; it is usually of plant or animal origin. (Ezeronye, 2007). Food consists of chemical compounds which heterophilic living thing consumes in order to carry out metabolic processes. They are also substances which when introduced to the digestive system under normal circumstances contribute to growth, repair and production of energy (Ezeronye, 2007). Ready-to-eat foods also described as street vended food are prepared and/or sold by vendors in the street or market for immediate consumption or consumption at a later time without further processing or preparation (Mensah *et al*., 2002). There are three categories of street vended foods (Chakravarty and Canet 1996); food prepared in small factories and sold by mobile vendors; food prepared at mobile vendors home and offered for sale to the public; and food prepared and sold on the street. Street foods increased consumption has also been attributed to their significant contribution to nutrition and food security for millions of practitioners along the food chain (Akinleye 1998) as well as being identified as potential vehicles for micronutrient fortification. These foods have for a long time now been praised in many developing economies for offering various business opportunities to small-scale entrepreneurs. Though popular, street food vending activities in most developing countries are mostly outside the regulation and protection of the governments (Alimi, 2016) and official volume of trade involved is largely lacking. (Alimi and Workneh, 2016) The diversity of street foods is extensive, as they vary widely not only from country to country, but also from vendor to vendor (Modarressi and Thong, 2010). Street foods are prepared in many ways: they can be fried, roasted, boiled, baked, steamed or eaten raw, depending on different cultures. (FAO/WHO, 2005) Different kinds of food products are vended in different parts of the world.

In Nigeria, the common street vended foods includes; fried meat, fried bean cake, fried fish, suya, fried yam, kulikuli, groundnut, kunnu and zobo among many others. Even with their perceived nutritional benefits to the society, street-sold foods are considered among the contributors of foodborne diseases e.g. they can transmit pathogenic microorganisms that can cause illnesses (Estrada-Garcia *et al*., 2004; Muyanga *et al*., 2011). The presence of chemical hazards such as pesticide residues, heavy metals and process contaminants can also contribute to foodborne illnesses. Thus, their quality is of great food safety concern as the consumers are continuously exposed to the risk of getting ill. (Zeki *et al.*, 2015).

Food-borne diseases are defined as diseases of infectious or toxic nature which are caused by the consumption of food or water (Kadariya and Thapaliya, 2014). Intoxication (toxin produced by the pathogens causes food poisoning), infection (ingestion of food containing pathogens), and toxicoinfections (producing toxins while growing in the human intestines) are the three types of food-borne diseases (Dharma *et al*. 2013; Addis and sissy; 2015) Food products may become contaminated at different stages along the food chain (Heredia and Garcia, 2018), could be during production, processing, distribution, preparation, and/or final consumption. In developing countries a major source of ready - to - eat foods are prepare and or sold at public places such as markets place, schools, canteens and along the streets, all together termed street foods (SFS). The ready-to-eat food are offered at relatively cheaper cost and at easily accessible places. Furthermore, it offers the traditional meals and preparations of a number of them that are quite laborious and time consuming. (Amoah, 1992; Chakravarky and Canet, 2002). However, a number of observational studies have shown that these foods are sometimes held at improper temperature, excessively handled by food vendors and sold at very dirty surrounding (WHO, 2001; Ghosh *et al*., 2007). In addition inadequate food safety laws, weak regulatory systems,

lack of financial resources to invest in safer equipment, and lack of education for food handlers are the reasons for common occurrence of food-borne diseases in developing countries (Haileselassie,2013; Ayana *et al*., 2015; Kebede *et al*., 2014). The microbiological quality of food indicates the amount of microbial contaminants it has; a high level of contamination indicates low quality and more likely to transmit infection. The concerns have been raised by the food and Agricultural organization (FAO) and others about these foods serving as a potential source of food poisoning outbreaks (Chakravarty and Canet, 2002).

Foodborne Illnesses of microbial origin are a major health problem associated with street foods (Rane2011; Kharel *et al*., 2016). Food-borne microbes are major problems affecting food safety and cause human infections after consumption of microorganisms or their toxins. In recent years, food-borne pathogens has become an important public health problem worldwide, and their impact on health (significant morbidity and mortality rate) and economy is increasingly recognized (Heredia and Garcia,2018; Zhao *et al*., 2014; Akbar and Kumal-Anal 2012; Bedasa *et al*., 2018). Foodborne bacterial pathogens commonly detected in street vended foods in developing countries include *Bacillus cereus, Clostridium perfringens, Staphylococcus aureus* and *Salmonella* spp. (Rane, 2011) and fugal pathogens include *Aspergillus niger, Fusarium* spp*, saccaromyces* spp. and *Penicillium* spp. These pathogens, among others, may result in foodborne infections and intoxications once contaminated food is ingested by the unsuspecting consumers. Street foods are often prepared by hand which may lead to an increased incidence of contamination with the potential foodborne pathogens. (Rane 2011; Kharel *et al*., 2016). The risk of contamination usually varies with the type of street food and how the food is prepared. Microbiological foodborne diseases have been widely reported to immensely influence the economies of both developing and developed countries in a negative way, which has necessitated

street foods safety to remain a principal priority for most governments (Ekanem, 1998), also concerns have been raised by the Food and Agricultural Organization (FAO) and others about these foods serving as a potential source of food poisoning outbreaks (Chakravarty and Canet, 2002). The contamination of these ready-to-eat food indicates low quality of food though the initial microbiological load of Ready-to-eat food ingredients is important, however, factors such as handling, processing, storage and display may influence the microbiological load of Ready-to-eat foods at the point of sale (Beuchat and Ryu, 1997; Angelidis *et al*., 2006) by the fact that many street vended foods in developing countries are prepared under unsafe environmental conditions such as close to municipal waste disposal sites that provide favorable breeding sites for insects and rodents which can easily contaminate the foods. In most cases also, basic provisions such as running potable water, washing facilities, toilets and organized waste disposal are often unavailable at the retail sites (Muyanga *et al*., 2011) which can put consumers health at risk of microbial foodborne illnesses. (WHO, 2002). In addition, the vendors practice poor personal hygiene and reports of food vendors being carriers of pathogens who eventually transfer these food borne hazards to consumers and therefore could serve as a potential source of transmission of many other illness to consumers. Most of the vendors have either no formal education or few years of schooling and therefore, lack knowledge on proper food handling and their role in the transmission of pathogens (Mensah *et al*., 2002). All of these increases the risk of street food contamination (Bhaskar *et al*., 2004, Tambekar *et al*., 2009) ranging from the initial contamination of raw foods with pathogens and to subsequent contamination by vendors during preparation, post production and handling of leftover. Thus there are many factors that should be considered for assessing the quality and safety of street foods (Mankee *et al*., 2003).

1.1 AIMS/OBJECTIVES

The aim of this research was to isolate, enumerate and characterize fungal contaminants present in fried bean cake (Akara) sold in four Local Government Area (L.G.A) across the Benin Metropolis. The specific objectives of this research were to:

1. isolate and identify the fungal isolates from fried bean cake (Akara) obtained across Benin Metropolis
2. enumerate fungal isolates from *fried bean cake (Akara)* obtained across Benin Metropolis
3. determine the frequency of occurrence of the fungal isolates
4. determine possible predisposing factors and then proffer valid recommendations on the findings.

CHAPTER TWO

LITERATURE REVIEW

2.0 BEANS

Beans is an edible nitrous seed of an erect or climbing plants (as of the general *phaseolus* and *Vigna*) of the legume family. Beans is a common name for large seeds of several genera of the flowering plants family Fabaceae (also know as *Luguminosae*) which are used for human and animal food. International institute for Tropical Agriculture reported that cowpea (*Vigna unguiculata*) is one of the most common varieties of beans; cowpea is a good animal feed crop grown in the semi or tropics covering Africa, Asia and Europe. Cowpea is a rich source of protein which belongs to the class of legumes, are often referred to as "Poor man's meat" due to their use as primary protein sources (Henshaw and Sobowale, 1996; Odedeji and Oyeleke, 2011). It is an important dietary staple in West African countries because of its high nutritional value, low cost and broad availability in the region. Generally cowpea contains 11% moisture, 24% protein, 1.3% fat, 56.8% carbohydrate, 3.9% fiber, 3.6% ash, 343 Kcal/100g (Deshpande and Damodaran, 1990) and also provides other nutrients such as calcium, magnesium, iron, potassium and zinc (Aykroyd *et al*., 1982; Uzogara and Ofuya, 1992). The plant tolerate drought, performs well in a wide variety of soils and been a legume replenishes low fertility soil when the roots are left to decay. It is grown by small scale farmers in developing countries where it is cultivated with other crops as it tolerates shades. It also grows and cover the ground quickly, preventing erosion. Cowpea's high protein content, its adaptability to different soil and intercropping systems, ability to improve soil fertility and prevent erosion makes it is an important economic product in many developing regions. All parts of the cowpea crop are used,

as all are rich in nutrients and fibers, its consumption cuts across different social and economic strata and its popularity is spreading to other parts of the world too. Most of the world's cowpea production is produced in West Africa, where cowpeas are eaten on a daily basis in other foods like soups, Akara and moin-moin (steamed cowpea paste). The consumption of cowpea is especially important in Nigeria since malnutrition is a major problem in this area of the world. With the increasing prices of animal protein, legumes continue to provide a cheaper source of protein than meats (Akinyele and Onigbinde, 1988).

2.1 OVERVIEW OF FRIED BEAN CAKE

Akara is a popular ready to eat food in Nigeria and other West Africa Countries (Ngoddy *et al*., 1986; Henshaw and Lawal, 1993; Ekariko, 2005) and forms part of diet for most ethnic groups in Nigeria. Its consumption cuts across different social and economic strata. It is often consumed alone or along a number of starch based foods such as garri (fried fermented grated cassava that is soaked in water), solid or liquid "ogi" (boiled fermented milled corn), bread and fried yam. Nigerians usually eat it as breakfast with "ogi", as lunch with garri or even dinner with "eko". It is considered to be a commonly consumed cowpea (*Vigna unguiculata*) based food in West Africa (Henshaw and Lawal, 1993; Aware *et al*., 2013). This savory finger food is made from whipped cowpea paste seasoned with chopped fresh pepper (either hot or mild), chopped fresh onion and salt which is fried in palm oil or vegetable oil at about 193°C.

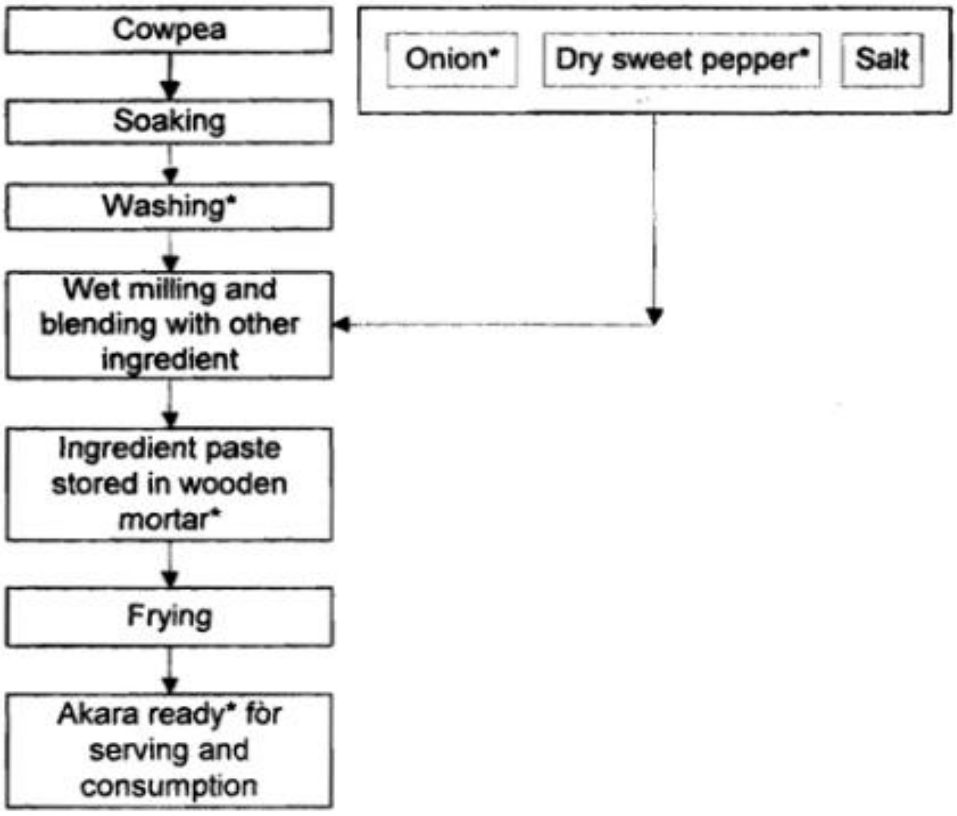

Figure: A flowchart showing procedures for preparation of fried bean cake

Akara like any ready-to-eat food requires inspection at different stages; from raw materials to finished products to ensure safety standards are met. Such a product may become microbiologically contaminated from its raw material, handlers, equipment and packaging material (Jay, 1987). The raw material (copwea) is a legume and are subjected to various contaminations during harvest or storage among which are those caused by mycotoxins which are produced by toxigenic molds of the genus *Aspergillus, Penicillium* and *Fusarium* (Zinedine and Idrissi, 2007). Microorganisms are ubiquitous in the environment and when there is neither proper sanitary practices during processing nor hygienic display of the product as well as satisfactory packaging materials for the finished product there is possibility for the product being contaminated constituting a risk to public health. The implications of consuming contaminated fried bean cake (Akara) is the risk associated with ingestion of microorganisms and other allergens produced by these contaminants.

2.2 SOURCES OF HAZARD IN PREPARATION OF FRIED BEAN CAKE

Hazardous foods defined as food in a form or state, which is capable of supporting the rapid and progressive growth of infectious and/or toxigenic microorganisms. According to Lateef *et al* (2010) it was observed that both microbial and chemical hazards can be encountered during various stages of fried bean cake production. The chemical hazards are encountered during procurement of beans in the form of residual pesticides, water (through improper storage in rusty metallic containers) and heavy-metal contamination from the grinding machines. The microbial hazards in the form of vegetative pathogens and spores can be encountered through the use of non-portable or contaminated water, grinding, use of bare hands, low level of sanitation and

personal hygiene and poor manufacturing practices such as cooling in uncontrolled environment, poor storage conditions and lack of use of basic equipment.

2.3 MICROORGANISMS IMPLICATED IN CONTAMINATION OF FRIED BEAN CAKE

The variation in the type of microorganisms isolated from fried bean cake *(Akara)* by different authors is attributable to the microflora associated with the cowpea, water, processing equipment and handlers, which may vary widely. Also, during the stages of cooling, which is usually done in an open space, varying degrees of handling and re-packaging, including those by children of low hygiene and inadvertent inoculation of the polythene bags by mouth-blowing to open them, introduces different level of contamination.

In a recent study on ready-to-eat foods, *E. coli, S. aureus* and *Bacillus* sp. were isolated from hawked fried bean cake *(Akara)* (Adegoke *et al*., 2008). *E. coli, Streptococcus faecalis, Bacillus subtilis* and *Klebsiella pneumoniae* from contact surfaces and ingredients during the production of fried bean cake *(Akara)* (Badau *et al*., 2001). Similarly, fungi were isolated from the various samples, but these are mainly Candida yeasts and *Aspergillus niger*. Some authors have previously isolated these fungal isolates from cowpea pastes and fried bean cake (Akara) samples (Bulgarelli *et al*., 1988; Badau *et al*., 2001). It has been observed that the most sensitive ingredients in fried bean cake (Akara) processing is water. This is used in washing, milling and preparation of the ingredients paste which is another sensitive ingredient. Sources of microorganisms could also be either from the contact surfaces (wooden mortar, milling machine and personnel hands), cowpea, onion and sweet pepper. Nkama *et al* (1994) isolated *Aspergillus fumigatus, Rhizopus arrhizus, Bacillus brevis* and *Bacillus alvei* from sweet pepper (tattashe) and

therefore could add to the microbial load of the ingredient paste. Other researchers have also isolated various bacteria, mould and yeast spp. from *fried bean cake (Akara)* ingredients pastes sampled from local markets in Nigeria (Bulgarelii *et al*., 1988). These are organisms that causes varying degrees of infections and poisoning in humans.

2.4 FUNGI IMPLICATED IN FRIED BEAN CAKE

Fungi are ubiquitous, eukaryotic microorganisms which are found in many different environments wherever organic material is available. Fungi are major spoilage pathogens of food and feedstuffs, the proliferation of various fungi and production of toxins in various food products leads to reduction in yield and quality with significant economic losses (Adejumo and Adejoro, 2014; Bankole, 1994; Bayman and Baker, 2006; Richard, 2007). Moulds are important in food because they can grow even in conditions in which many bacteria cannot grow, such win general, moulds are able to grow at lower pH of of 0.80 or lower and thus can grow on partially dehydrated surfaces (including food) and tend to be less thermophillic compared to bacteria and other microbes. They also produce secondary metabolites which are referred to as mycotoxins which have been found to be present in most food substances. The mycotoxins are low weight metabolites which cause harm known as mycotoxicoses, in live-stock, domestic animals and humans and therefore are of public health significance (Ashiq, 2015; Bayman and Baker, 2006; Bhat and Vasanthi, 2003; Jeswal and Kumar, 2015; Richard, 2007). The genera of fungi are mainly represented by *Aspergillus*, *Penicillium* and *Fusarium,* but *Trichoderma, Trichothecium* and *Alternaria* are also important as food contaminants or pathogens for plants, among others (Ashiq, 2015; Richard, 2007).

2.4.1 *Aspergillus*

Aspergillus is a group of moulds that is found worldwide and are commonly found in air, water, soil. It spoils a wide variety of food and non-food items (paper, leather, etc.) but are probably best known for spoilage of grains, cereal, tree nuts, and some spices. High temperature, high moisture, retarded crops and poor food storage conditions enhance the mold growth and mycotoxin development. Their potential for contamination of food stuffs is widespread under favourable environmental conditions. The genus *Aspergillus* is a large proportion of all the moulds found in industrial food (Adejumo and Adejoro, 2014; Onions, Allsopp and Eggins, 1981; Richard, 2007). They have particular importance as spoilage organisms of food. Changes due to spoilage by *Aspergillus* species can be sensorial, nutritional and qualita-tive nature like pigmentation, discoloration, rotting development of odours and oddor flavours. Many species grow at very low water activity and are found attacking various foods and producing mycotoxins (Adejumo and Adejoro, 2014; Lee, Wang, Allan, and Kennedy, 2004).

2.4.2 *Penicillium*

Penicillium is present in soils and plant debris from both tropical and Antarctic conditions but tend to dominate spoilage in temperate regions. They are distinguished by their reproductive structures that produce chains of conidia. Although they can be useful to humans in producing antibiotics and blue cheese, many species are important spoilage organisms, and some produce potent mycotoxins (patulin, ochratoxin, citreoviridin, penitrem). *Penicillium* spp. cause visible rots on citrus, pear, and apple fruits and cause enormous losses in these crops. They also spoil other fruits and vegetables, including cereals and grains. Many of *Penicillium* species can also produce a wide range of toxic compounds such as citrin and citreoviridin (Richard, 2007).

2.4.3 *Fusarium*

Fusarium is a filamentous fungus producing thread-like hyphae that enable it to penetrate plant surfaces and ramify through (colonise) host tissues as primary or secondary invaders. Fusarium is one of the most economically important genera of fungal causing significant crop losses and contamination of grain by mycotoxins on a global basis. Some species also cause infections (mycoses) of humans and other animals. They produce mycotoxins such as deoxynivalenol and zearalenone (Richard, 2007).

2.4.4 Yeast

Yeasts are widely distributed in nature. They thrive on plant leaves, flowers and especially fruits. They occur on the skin, hide, feathers and also in the alimentary tract of herbivorous animals. Some types of yeast are commonly associated within insects and can survive during unfavorable periods. These natural habitats are important vehicles for carrying yeasts into food.

2.5 SOURCES OF FUNGI IMPLICATED IN FRIED BEAN CAKE CONTAMINATION

The presence of *Mucor* sp, *Penicillium* sp., *Aspergillus niger, Aspergillus flavus, Fusarium* sp. and *Rhizopus stolonifer* in food sample is not surprising as they are dispersed in the form of spores which is abundant in the environment and can be introduce through dust and soil (Apinis, 2003).

The presence of *Aspergillus, Penicillium* and *Mucor* could be attributed to the surrounding air and packaging materials (Aboloma *et al*., 2008; Kawo *et al*., 2009).

Aspergillus niger are commonly found in indoor environment and can easily contaminate the environment in food processing areas which can spread onto food and cause contamination (Samson *et al*., 2001).

Aspergillus, Aphanomyces, Curvularia, *Fusarium, Penicillium* and *Rhizopus* species have been reported to be associated with foliar and root diseases of beans, peas and other legumes (Abdulwahab *et al*., 2015). They successfully colonize the grains and alter its nutritional properties (Samson, 2017). Some of the species of these fungi produce toxins such as the aflatoxins, which can be carried over to the derivative products.

Additionally, seeds damaged by insects could serve as a port of entry for fungal infections and toxin production (Dennis, 2002) thereby helping their rapid spread.

Fusarium, Mucor sp, *Aspergillus fumigatus, Aspergillus niger*, *Saccharomyces cerevisiae* and Rhizopus, isolated from the beans flour, indicates that microorganisms in raw legumes can be carried into its processed derivative (Badmos *et al*., 2012).

Also, persistent exposure of food products when on sale could have contributed immensely to the presence of spores from fungi species in food product (Badmos *et al*., 2021). Due to the weather conditions in the tropics where temperature and relative humidity is high the proliferation of these spores in food is a certain occurrence.

2.6 MYCOTOXINS IN FOOD

The toxins, which are secondary metabolites, are produced by filamentous fungi which may contaminate foods at any stage during production and storage (El-bouhy *et al*., 1993) causes different foodborne diseases. The major mycotoxin of food safety importance are Aflatoxin, Ochatoxin, Citrinin, Deoxynivalenol and Zearalenone. Most of these microbial toxins are thermostable in nature and are not destroyed by high temperatures during cooking or food processing (Rajkovic, 2014). Within normal food processing temperature range (80ºc-121ºc), there is little or no destruction of these toxins and they are therefore said to be heat-stable. Therefore, under normal cooking conditions, such as frying and boiling or even following pasteurization; these toxins remain active (Milicevic *et al*., 2010). The production of mycotoxins by fungi is stimulated by certain environmental factors: therefore the extent of contamination will differ with geographic location, agricultural methods and the susceptibility of commodities to the penetration of fungi during storage and processing periods (Jonathan and Esho, 2010). These toxins have attracted public health concerns owing to their nephrotoxicity, teratogenicity, immunotoxicity, etc. (Bhat and Vasanthi, 2003). Although inhalation and dermal contact can also expose one to mycotoxins and causing most cases of mycotoxicoses in animals and humans occur through ingestion.

2.7 FOOD BORNE DISEASES

According to different reports, a huge number of people suffer from food-borne diseases each year worldwide (Ejo *et al*., 2016), and around 600 million (10 people in the world) become ill due to the consumption of contaminated food (Guerra *et al*., 2016). Due to unrecognized or unreported outbreaks, statistical data of food-borne diseases are increased (Heredia and Garcia,

2018). Food-borne diseases are major health problems both in developed and developing countries (Abunna, 2016), but developing countries tend to suffer from the largest share of the burden of food-borne diseases (Ayana *et al*., 2015). According to the WHO, 30% of the population suffer from food-borne diseases each year in developed countries, and up to 2 million deaths are estimated per year in developing countries (Abunna *et al*., 2016) due to Prevailing poor food handling and sanitation practices.

Several bacterial isolates, namely, *Escherichia coli, Staphylococcus aureus, Staphylococcus epidermidis, Citrobacter freundii, Serratia marcescens, Proteus vulgaris, Bacillus cereus, Streptococcus pyogenes, Bacillus* sp. and *Shigella* sp. and fungal isolated such as *Aspergillus niger,* yeast and mould were detected in contaminated Akara samples all of which can cause varying diseases conditions which include: gastroenteritis, shigellosis, cholera and even liver and kidney diseases.

2.8 RECOMMENDED SAFETY MEASURES IN FOOD

Waste disposal is another factor that presents risk of food contamination. Food hygiene requires that refuse or filth must not be disposed near the place where food is being processed and must not be allowed to accumulate or be left in food rooms. Enough space should be provided in a suitable part of the premises for separating sound food from unsound food and for storing waste while it awaits disposal.

Liu (2007) observed that spores of some pathogenic organisms such as clostridium botulism are present in dust in food preparation areas and that all types of food poisoning bacteria can be

spread by cross contamination. It is therefore very important that dust should be removed daily from the cooking areas to avoid food contamination.

According to the Federal Ministry of Water Resources (2000), it has been estimated that as many as 80% of all disease in the world are associated with unsafe water or poor environmental hygiene. Unsafe water is used not only for drinks in various forms, but also in food preparation and cleaning operation. The pathogen enters the body through contaminated drinking water or through food which has been contaminated via water hence the need for safe water becomes very important.

Damaged grains should be sorted and eliminated to avoid contamination with mycotoxigenic organisms (Badmos *et al*.,2021)

Also, stringent laws on raw legume grains should be incorporated and enforced in order to reduce the spread of aflatoxins in food samples (Badmos *et al*.,2021)

Recent evidence suggests that some true *Aspergillus niger* strains do produce ochratoxin (Samson *et al*, 2004) proper sanitation in the kitchen and its environs is this important.

Proper and regular hand washing, sanitization of all equipment and utensils, care for the environment and the packaging materials so as to prevent the spread of contaminants will help in safety of food (Oranusi *et al*., 2011).

Handling of product with bare hand displaying it in open bowls and buckets during sales should be avoided to prevent introduction of fungi spores to product (Ebidor *et al*., 2015).

It is important to develop a strategy to properly package and store food products to reduce fungal contamination (Ebidor *et al*., 2015).

The general public should be educated on the need for food safety and the requirement for water meant for human consumption and for food processing (Taulo *et al* 2008; Okonko *et al*., 2008a and 2008b).

Some hazards can be abated using a number of control measures that bother on the use of high quality raw materials, maintenance of high level of hygiene, adoption of GMPs and use of basic equipment (lateef *et al,* 2010).

2.9 FOOD HYGIENE

Food hygiene is defined as a sanitary science which aims at producing food which is safe for human consumption and of good keeping quality and this includes any sanitation measures designed to prevent bacteria and other microorganisms of human origin from reaching food stuff (Umoh and Odibo, 1999). Food hygiene is a subject of wide scope, it aims at studying methods for production and preparation of food, which is safe and of good quality. It covers not only the proper handling of every variety of food stuff and drinks, but also food contact surfaces such as utensils, and apparatus used in the preparation, services and consumption of the food and also the care to prevent contamination with food poisoning bacteria which may originate from the animal or part plant host supplying the food (Umoh and Odibo, 1999).

CHAPTER THREE

MATERIALS AND METHODS

3.1 STUDY AREA

The study was conducted between the month of January and February, Ready-to-eat fried bean cake (Akara) from different vending sites across four (4) Local Government Areas in Benin Metropolis were sampled. These vending sites were chosen because they are highly patronized by members of the general public of different socio economic classes (upper, middle and lower class).

3.2 SAMPLE COLLECTION

Twelve (12) samples of fried bean cake (Akara) was collected from 4 Local Government Area (Ikopha Okha, Oredo, Ovia North-East and Ovia) in Benin City, Edo state and the samples were immediately transported to the laboratory for fungal analysis. The study was carried out between the month of January and February, 2022 at Mycofarm Laboratory, Benin City, Edo state.

3.3 STERILIZATION OF MATERIALS

All glassware (test tubes, conical flask, glass spreader and measuring cylinder) were sterilizad in a steam oven at 121°C for 15 minutes before use and then allowed to cool down at room temperature before usage. Agar media used were sterilized at 121°C and 15psi for 15 minutes in an autoclave. All media used were freshly prepared and refrigerated at 3-4°C. The entire

working surface was also disinfected with ethanol to reduce contamination. Aseptic conditions were ensured during innoculation and sub-culturing.

3.4 PREPARATION OF CULTURE MEDIA

Potato Dextrose Agar (PDA) was used for the enumeration of the fungi as well as in the pure culture selection of the organisms; the media was prepared as instructed by the manufacturers.

3.4.1 Potato Dextrose Agar

The medium used for isolation of fungi was Potato Dextrose Agar (PDA). This was prepared according to manufacturer's instruction. About 36.9g of powdered PDA was dissolved in 1 litre of sterile distilled water and sterilized at 121°C for 15mins. After cooling to about 45°C-50°C it was then aseptically dispensed into sterile Petri dishes.

3.5 SAMPLE PREPARATION

Aseptically, 1g of each sample was macerated into bits using a sterile laboratory mortar and pestle.

3.6 SERIAL DILUTION

One gram (1g) of each sample was dispensed into a prepared 9ml of distilled water contained in the McCartney bottles. The content was shaken for homogenous mixture. This produces a

homogenate used as a stock solution of each sample. One in ten serial dilutions (10^{-1} – 10^{-3}) of the samples were then prepared.

3.7 POUR PLATE

Fungal culturing was made using the poor plate technique. Enumeration of total fungal count was also done using Potato Dextrose Agar (PDA). An Antibacterial agent (Streptomycin) was added to prevent bacterial growth.

3.7.1 CULTURING OF SAMPLES

0.1ml of inoculums from the 10^{-3} dilution tubes was transferred aseptically to appropriately labeled Petri dish. Streptomycin was added to prevent bacteria growth. Potato Dextrose Agar was used for fungal culture. The plates were incubated at room temperature for 3-5 days.

3.7.2 PURE CULTURE

To obtain a pure culture, each distinct fungal colony was sub cultured on freshly prepared Potato Dextrose Agar plate using streaking method and the plate were incubated at room temperature for 48-72hrs for subsequent taxonomic identification.

3. 8 ENUMERATION AND ISOLATION OF MICROORGANISM

The discrete colonies on the Potato Dextrose Agar (PDA) were counted. The total fungal count at the dilution factor 10^{-3} were used to estimate the total viable count for the samples in colony forming units per gram(cfu/g).

Total fungal count (cfu/g) = Number of colonies

$$\frac{\text{}}{\text{Volume of aliquot} \times \text{Dilution factor}}$$

3.9 CHARACTERIZATION AND IDENTIFICATION OF FUNGAL ISOLATES

Fungal isolates were identified based on cultural and morphological characteristics.

3.9.1 CULTURAL CHARACTERISTICS

The physical characteristics of the mycelia such as the colour, form and margin were noted.

3.9.2 MORPHOLOGICAL CHARACTERISTICS

The fungi were identified using lactophenol cotton blue technique. In this process, a drop of lactophenol cotton blue was placed on the grease free sterilized slide. A sterile wire loop was used to pick the organism from the colony and evenly spread on a slide then covered gently using a cover slip then allowed to stay for some seconds and then examined under a microscope (x40 objective lens) to check for the colonial and morphological characteristic of each isolate. Some morphological structures employed for identification include septation, presence/absence of sporangiophores, fruiting bodies and other special organs like the rhizoids

CHAPTER FOUR

4.0 RESULTS

Table 4.1: Shows the mean fungal count of fried bean cake (Akara) samples from three different vendors across four Local Government Area around Benin metropolis. The highest mean count of 3.7x 10^4 was recorded in Ovia N/E and Ikpoba Okha Local Government Area and the least mean count of 1.3x 10^4 in Egor Local Government Area.

Table 4.1: Mean count of fungal isolates from fried bean cake (Akara) across four (4) Local Government Area in Benin metropolis

Location	**Sample 1**	**Sample 2**	**Sample 3**	**Mean count (Cfu/ml)**
Ovia N/E	$2x10^4$	$4x10^4$	$5.0x10^4$	$3.7x10^4$
Egor	$2x10^4$	NG	$2.0x10^4$	$1.3x10^4$
Oredo	$1x10^4$	$5x10^4$	$3.0x10^4$	$3.0x10^4$
Ikpoba okha	$1x10^4$	$3.0x10^4$	$7.0x10^4$	$3.7x10^4$

Table 4.2: Shows the cultural (in Potato Dextrose Agar) and morphological characteristics of fungal isolates from four different Local Government Area around Benin metropolis. The table shows the suspected microbes which are; *Penicillium chrysogenum, Aspergillus flavus, Fusarium oxysporium, Mucor mucedo* and *Saccharomyces* sp.

Table 4.2: Cultural and morphological characteristics of fungal isolates from fried bean cake (Akara) across Four(4) Local Government Area in Benin metropolis

Cultural	Green flat colony with white periphery	Greenish yellow colony	Cottony white colony with reverse side dirty white	White flat colony with reverse side colourless	Medium creamy with convex elevation and entire margin
Morphological					
Nature of hyphae	Septate	Septate	Septate	Non-septate	Pseudohyphae
Colour of spore	Green	Coffee black	Army green	Colourless	Colourless
Type of spore	Conidiophore	Conidiophore	Conidiophores	Sporangiosphore	Chlamydosphore
Appearance of special structure	Brush-like conidia	Branching conidia	Unbranching conidia	Sporangium	Budding
Fungal isolates	*Penicillium chrysogenum*	*Aspergillus flavus*	*Fusarium oxysporium*	*Mucor mucedo*	*Saccharomyces* sp.

Table 4.3: Shows the prevalence of fungal isolates from different vendors in four Local Government Area across Benin metropolis. It was deducted that *saccharomyces* sp had the highest frequency of occurrence 4(33.33%) while *mucor mucedo* had the least frequency of occurrence 1(8.33%).

Table 4.3: Shows the distribution and prevalence of fungal isolates from fried bean cake (Akara) across four Local Government Area in Benin metropolis

Fungal isolates	Locations				Occurrence(%)
	Ovia N/E	Egor	Oredo	Ikpoba Okha	
Penicillium chrysogenum	+	-	+	-	2 (16.66%)
Aspergillus flavus	+	-	+	+	3 (25.00%)
Fusarium oxysporium	+	-	-	+	2 (16.67%)
Mucor mucedo	-	-	+	-	1 (8.33%)
Saccharomyces sp.	+	+	+	+	4 (33.33%)

Keys:

\+ (Presence)

\- (Absence)

CHAPTER FIVE

5.0 DISCUSSION

Microbiological sources of food contamination are more preponderant and are therefore of greater concern than other sources of contamination such as chemical and physical sources because of the quantum of illness associated with it (Scallan *et al*., 2011). WHO (2007) estimated that a significant proportion of the approximately 1.5 billion episodes of diarrhea and more than 3 million deaths globally recorded annually, results from consumption of food with microbial pathogens and toxins therefore, microbiological considerations are of paramount importance. Fungal count is very important in determining the level of mould/yeast contamination which connotes the likely presence of Aflatoxin (mycotoxin) producing fungi (Nkama, 1987). This study therefore aimed to enumerate, isolate and characterize fungal contaminants in fried bean cake (*Akara*) sold across Four Local Government Area in Benin metropolis. In this study, *Aspergillus flavus, Fusarium oxysporum, Penicillium chrysogenum, Saccromyces* sp., and *Mucor mucedo* were isolated from the Akara sample collected from the four different Local Government Area across Benin City, Edo State. Various researchers have previously isolated these fungal isolates from cowpea pastes and the fried bean cake (Akara) samples (Bulgarelli *et al*., 1988; Badau *et al*., 2001). Akara samples from Ovia North-East and

Ikopha Okha had the highest mean fungal count (3.7×10^4 cfu/g) while samples from Egor had the least fungal count (1.3×10^4 cfu/g). *Saccromyces* sp. had the highest frequency of occurrence (33.33%) while *Mucor mucedo* had the least frequency of occurrence (8.33%). Although the sample does not exceed the mean microbial count recommended standard of 105 cfu/g for ready-to-eat foods (Okeke *et al*., 2014; Jorgensen *et al.*, 2017) and the total fungal count of 10^4 cfu/g for food (Amadi *et al.,* 2014) there might have been hygienic problems either in the processing or handling of the food.

Results from this study indicated that *Saccromyces* sp. was most prevalent. This could be attributed to the natural yeast populations in raw ingredients for Akara processing and contact surfaces (Badau *et al*., 2001). Generally, it has been observed that the initial micro flora of the ingredients paste and those found on the contact surfaces were source of Akara contamination. (Badau *et al*., 2001). *Aspergillus flavus* was the second most prevalent fungus and its presence in food may be hazardous to public health. *Aspergillus* spp are very common fungal agent of food borne illness, (Peraica and Domija, 2001). *Aspergillus* species some of which are known to produce Aflatoxin causes *Aspergillosis* in patients with weakened immune systems (Adebayo-Tayo *et al*., 2012b). Their occurrence may be attributed to their spores that are widely dispersed in the environment and are heat resistant thus survive high temperature which agrees with the work of Oranusi *et al*. (2013) that microbial spore mostly contaminate food from dusty environment and heat-resistant spores could survive temperature that can destruct vegetative cells. *Fusarium oxysporum* is a fungal contaminants of legumes and were isolated from fried bean cake (Akara) samples which agrees with Temba *et al*., (2017) who reported that certain fungi are found in legumes and legumes based food. The colonization of food by fungi species is a process that takes place from farm to fork. Also, cowpea itself would contribute to the

microbial load observed in the pastes, as a result of microflora such as *Aspergillus flavus* and *Fusarium oxysporum* that are associated with it**.** Therefore, the practices of sorting and drying in order to have good quality of grains with low moisture content for storage applied by the farmers before the storage should contribute to reduce the risks of cowpea infection by fungi. Indeed, these operations have been reported by Bankole and Adebanjo (2003) as been a good and advisable practices against the infection and the development of fungi on grains in stock. In addition, the rotten grains and other undesired foreign material can be found in harvested cowpea and when used for processing it can be transferred to its processed derivative (Badmos *et al*., 2021). *Mucor mucedo* had the least frequency of occurrence. Mould disperse in the form of spores and it is abundant in the environment and can be introduced through dust and soil as these vendors are mostly situated along road sides. The presence of *penicillium* sp. could also be attributed to the presence of its spores in the surrounding air as well as packaging materials. Infections caused by *Penicillium* sp. include rhinocerebral mucomycosis, genitourinary, mucocutaneous, gastrointestinal, pulmonary and disseminated infections (Odu and Maduka, 2019). Other factors that might have contributed to the level of contamination include carry-over effects as regard utensils, containers and milling machines. Food vendors are also potential source of cross-contamination to food product as they may carry fungi spores on themselves and their hands.

5.1 CONCLUSION

The result of this study showed that fried bean cake (*Akara*) samples collected from four different Local Government Area across the Benin metropolis were contaminated with one or more fungi species. These contaminants are encountered due to poor hygiene in processing,

handling and distribution of food product therefore poses as serious health hazard. Thus, there is need to emphasize the need of food hygiene, enlightenment of food vendors on food safety precautions, as well as the enforcement of strict laws to ensure that food safety measures are followed.

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