

**ISOLATION AND CHARACTERIZATION OF FUNGI IN OSE-OJI (GROUNDNUT
SAUCE) IN SOME LOCAL GOVERNMENT AREAS IN EDO STATE**

BY

Chelsy Etinosa IYAMU (MISS)

LSC1609444

DEPARTMENT OF MICROBIOLOGY,

FACULTY OF LIFE SCIENCE,

UNIVERSITY OF BENIN,

JULY, 2021

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**A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY,
FACULTY OF LIFE SCIENCE, UNIVERSITY OF BENIN, BENIN CITY, IN PARTIAL
FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF DEGREE OF B.Sc
(HONS) IN MICROBIOLOGY**

JULY, 2021

CERTIFICATION

This is to certify that the research project work was done by **Chelsy Etinosa IYAMU (MISS)** in the Department of Microbiology, Faculty of Life Sciences, University of Benin under my supervision.

Mr. E. I. Obazenu
(Project Supervisor)

DATE

PROF. S. E. OMONIGHO
(Head of Department)

DATE

APPROVAL

I certify that this report was accepted in partial fulfillment of the requirement for the award of
B.Sc. (Honours) in Microbiology.

PROF. S. E. OMONIGHO
(Head of Department)

DATE

DEDICATION

This project work is dedicated to God Almighty for my existence and sustenance all through my life. Also to my parents.

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My profound gratitude and acknowledgement goes to my eminent and amiable supervisor and course adviser **Mr. E. I. OBAZENU** for correcting with love, patience and commendation throughout this project work thus making it a success.

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ABSTRACT

Safe food is a basic human right, despite food contamination with naturally occurring pathogenic microorganisms. Such pathogens cannot be detected organoleptically (seen, smelled or tasted), but can cause diseases of varying severity. Thus, food safety issues are of major importance to world health. The main objective of this study was to determine the fungi contaminants of groundnut sauce (*ose oji*) sold in Benin City. The Total heterotrophic fungi count (THFC) was used as important indicator of the microbial quality of *ose-oji* samples. A total of 10 samples from five vendors (in triplicate) were cultured for total heterotrophic fungi counts on two different fungi media. It was observed that the mean fungi count of the samples ranged from 0.20 to 4.05×10^3 cfu/ml on malt extract agar and 0.50 to 4.90×10^3 cfu/ml potato dextrose agar on agar. The result of the statistical analysis for table 1 and 2 showed that there was significant difference between total heterotrophic count of samples collected from different locations and using different media for heterotrophic fungi count. The morphological, cultural and biological characteristics of microbial isolates revealed the following fungi genera; *Penicillium chrysogenum*, *Aspergillus niger*, *Mucor mucedo*, *Aspergillus Flavus* and *Rhizopus* spp. The studied peanut butter sauce sold in Benin City is nutritive and could meet the dietary needs of the population when consumed with other foods. Indeed, these peanut butters constitute a valuable sources of protein, fat, crude fibre and minerals. However, the presence of toxigenic fungi as *Aspergillus flavus* and other fungi species in these the soup preparation highlight a potential public health problem. Therefore, the need to educate both sellers and consumers on processing, food handling procedures and personal hygiene would help to minimize fungal contamination.

CHAPTER ONE

INTRODUCTION

Safe food is a basic human right, despite many foods are frequently contaminated with naturally occurring pathogenic microorganisms. Such pathogens cannot be detected organoleptically (seen, smelled or tasted), but can cause disease of varying severity, including death, specially if the way they are conserved during exposition for sales provides conditions for those microorganisms to grow and reach considerable levels of contamination. Thus, food safety issues are of major importance to world health (WHO, 2000). The global incidence of food borne illnesses is difficult to estimate but it has been reported that in 2000 alone 2.1 million people died from diarrhoeal diseases. A great proportion of these cases can be attributed to contamination of food and drinking water (WHO, 2000). Illness resulting from the consumption of contaminated food has become one of the most widespread public health problems in contemporary society (Notermans et al., 1995). In Nigeria, as in many developing countries, street foods (SFs) are prepared and or sold at public places such as schools, markets places, along the streets. The SFs offer food at relatively cheaper rate and at easily accessible places (Mensah *et al.*, 2002; Oranusi and Braide, 2012). Furthermore, it offers the traditional meals and preparations of a number of them are quite laborious and time consuming. Thus, with the increase in the number of hours spent at work places by parents and schools, the importance of SFs in the human feeding is increasingly becoming very important among all socio-economic groups (Amoah, 1992; Chakravarty and Canet, 2002). A number of observational studies have shown that these foods are sometimes held at improper temperatures, excessively handled by food vendors and sold at very dirty surroundings (WHO, 2001, 2003; Muinde and Kuria, 2005; Ghosh *et al.*, 2007). In addition, the vendors practice poor personal hygiene and reports of food vendors being carriers

and therefore could serve as a potential source of transmission of enteric fevers are many. 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). At the same time, most people who use these food services are more interested in its convenience than the question of microbiological quality and hygiene. The microbiological quality of food indicates the amount of microbial contaminants it has, a high level of contamination indicates low quality of food storage and its handling and more likely to transmit infection and the reverse is true (Anonymous, 1988). Thus 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).

1.1 Aim and objectives

AIM

The aim of this study was to determine the fungal quality of *ose oji* (groundnut soup) sold around Benin city, Edo state.

Objectives of the study are to

1. to estimate the total fungal load present in the *ose oji* samples
2. to isolate the fungi species from *ose oji*
3. to identify fungi isolates

CHAPTER TWO

LITRATURE REVIEW

2.1. Background of the study

Groundnut (*Arachishypogaea*) is an annual herb whose chief, and remarkable characteristic is the production of fruits underground. The many cultivars fall naturally into two distinct botanical groups depending upon differences between them in their branching habit. The groups are regarded as distinct sub-species, the Virginia and the Spanish or Valencia. In Sudan, early maturing Spanish types are grown in rained areas of Western and Southern Sudan, while the Virginia types are grown in the irrigated areas of the central and eastern parts of the country. Groundnut is one of the leading agricultural crops of the world for the production of edible plant oil and protein (Adegokeet *al.*, 2004). In developing and under-developed countries, there is an urgent need for additional or new plant foods to meet the nutritional requirements of ever-increasing populations (Prakash and Misra, 1988). Large segments of the populations from these countries suffer from malnutrition (Akuboret *al.*, 2000). Popular legumes, such as cowpea, beans and groundnuts are consumed to complement the low protein contents of grain; animal proteins such as meat, milk and eggs are expensive and relatively difficult to acquire. However, for efficient utilization and consumer acceptance of these legume seed flours, studies of their desirable functional properties are important. Previous studies on the functional properties of flours have focused mainly on 4 popular legumes, such as spopular legumes, such as soybean, cowpea, pigeon pea, and mucuna beans (Giami, 1993; Oshodi and Ekperigin, 1989; Wolf, 1970; Yusuf and Adewuyi, 2003).

2.2. Chemical composition of groundnut seeds

The chemical compositions of the groundnut seeds have been studied by numerous searchers, who found variation in results. This variation has been attributed to the differences in seed variety, soil, climate, storage conditions and processing methods for oil extraction in case of the cake. Groundnut is valued for its high oil quality content which ranges from 44% to 56% with an average of 50% (Cobb and Johnson, 1973). The protein content in the groundnut kernel ranges from 16.2 to 36% (Cobb and Johnson, 1973; Kalil and Chughtai, 1983; Icrisat, 1986). The major seed proteins globulins (salt soluble) of the groundnut seed contains about 18.3% nitrogen, albumins (water soluble) and glutamines (acid or alkaline soluble). These proteins are used in several food products for their functional properties, such as emulsifying and foaming capacity, or for their nutritional properties (Natarajan, 1980; El-Zalakiet *al.*, 1996). Groundnut seeds provide a wide range of mineral elements which can supplement human dietary mineral requirements. It was reported that the groundnut seed contains primary elements such as calcium, magnesium, potassium, sodium, iron, and certain trace elements such as manganese, copper, zinc, and boron in appreciable amounts (Mc Waters and Cherry, 1982). Groundnut seeds are known to be an excellent source of certain vitamins, especially vitamins E, K and the B group. The groundnut seeds are also one of the richest sources of thiamin (B1) in plants (Savage and Keanan, 1994). Generally the whole groundnut seed is a source of fiber, iron, magnesium, phosphorus, niacin, folate and vitamin E, as well is flavones and phytoestrogen, and other antioxidant compounds (Goldberg, 2003). Groundnut seeds contain 25 – 30% protein, 45 -55% oil, with 100 iodine value, main fatty acid: oleic, linoleic and palmitic. It is used in the manufacture of margarine; contain Ca, Mg, P and as good source of vitamins E and B, Niacin, Riboflavin and thiamin. It poor in vitamin A. (Khidir, 2007). Groundnut contain high

percentages of oil (50–55%) and protein (25–28%) and low percentages of carbohydrates and ash (Grosso and Guzman, 1995). It has been observed that rainfed Sodari cultivar contained higher oil content (49.6%), but low in protein content (23.8%), while irrigated cultivars, Ashford have a higher oil content (54.8%) and lowest protein content (23.4%). The irrigated groundnut cultivars are good sources of oil and oleic acid, and have higher Oleic/Linoleic ratio, while rainfed groundnut cultivar were good sources of protein (Elshafie, 2001). Groundnut is a rich source of oil. However, due to increasing awareness among consumers about figure and health, low-oil groundnuts are now being preferred for confectionery (Owens, 1994). The fatty acid composition of groundnut oil is influenced by both environment and genotype. Groundnut oil is composed of as many as 12 fatty acids, of which about 80% is accounted for by only two fatty acids viz. oleic and linoleic acids (Savage and Keenan, 1994). Tocopherols, derivatives of vitamin E, are lipid soluble natural antioxidants produced only by plants. Groundnuts contain approximately 26–60 mg tocopherols/100 g, of which 12–25 mg is α -tocopherol, 10–34 mg β -tocopherol and 0.6–2.5 mg γ -tocopherol (Cobb and Johnson, 1973). Mature groundnut kernels were reported to contain 9.5–19% carbohydrates in which starch and sucrose are the major constituents. Jiang and Duan (1994) reported that mean oil contents of seeds was ranged from 50 to 57% and mean protein content of seeds was 27.45%. Peanut oil generally contains 55–65% monounsaturated fatty acids, 26–28% polyunsaturated fatty acids, and 17–18% saturated fatty acids (Ory et al., 1992). Groundnut cakes contain in 954 g/kg dry matter, 79.6 oil g/kg 435.8 g/kg crude protein, 97.2 g/kg fiber, 92.5 g/kg ash, 6.2 g/kg Ca, 5.6 g/kg P, 5.3 g/kg Mg, 1.5 g/kg NaCl, 18.7 mg/kg Cu, 33.0 mg/kg Zn and 2.2 mg/kg Mn (Sulieman and Mabrouk, 1999). Other authors reported that groundnut cakes contain 95.11 g/100g dry matter, 8.77 g/100g oil, 55.3 g/100g protein, 10.25 g/100g fiber and 8.12 g/100g ash. (Batalet al, 2006) reported that, the

composition of peanut cakes showed a crude protein ranged from 40.1 to 50.9%, with a mean of 45.6%. Lysine, 1.54%; methionine, 0.52%; cystine, 0.64%; threonine, 1.17%; and arginine, 5.04%. Mean values for fat, fiber, and ash were 2.5, 8.3, and 5.0%, respectively. Average levels of calcium, phosphorus, sodium, and potassium were 0.08, 0.57, 0.01, and 1.22%, respectively. (Khidir, 2007) reported that, the groundnut contain 40 – 50% protein, rich in lysine and tryptophan and it was poor in methionine and cystine.

2.3. Functional properties

Functionality of food proteins is defined as those physical and chemical properties, which affect the behavior of proteins in food systems during processing, storage, preparation and consumption (Fennema, 1996). Vegetable oil extraction from groundnut yields partially defatted groundnut flour (DPF). DPF is a protein-rich, inexpensive and underutilized by product of the groundnut industry that offers the same health and dietary benefits of groundnut with less fat. DPF contains 47–55% high quality protein with high essential amino acid content (Basha and Pancholy, 1982; USDANAL, 2005) and lends itself being used in many food applications (Prinyawiwatkul *et al.*, 1993). Defatted groundnut flour despite having an excellent amino acid profile, has found only marginal use in food industry due to its inferior functional properties (Beuchat *et al.*, 1975). So the development of a new protein product such as peanut protein concentrate (PPC) from defatted groundnut flour would be more important, for it could provide the food industry with a new high protein (more than 70 g protein/100 g product) food ingredient for product formulation and protein fortification (Wu, *et al.*, 2007). The latter is critically needed in many developing countries, because animal protein is more expensive and is getting beyond the reach of many people in developing countries. Abundant proteins in peanuts are cheaper sources of proteins that would serve the purpose. Functional properties of food proteins are

important in food processing and food product formulation. Some of these properties are water holding/oil binding, emulsification, foam capacity and gelation. These properties are affected by the intrinsic factors of protein such as molecular structure and size, and many environmental factors including the method of protein separation, production, pH, ionic strength and the presence of other components in the food system. The importance of these properties varies with the type of food products in which the protein concentrate is used. For example, proteins with high oil and water binding were desirable for use in meats, sausages, bread and cakes, while proteins with high emulsifying and foaming capacity were good for salad dressing, sausages, soups, confectionery, frozen desserts and cakes (Ahmedna *et al.*, 1999).

Functional properties of protein are influenced by many factors. For end users, pH, temperature and ionic strength of the food systems are important factors to consider. For producers, methods and conditions of protein extraction, as well as downstream processing of extracted protein such as purification and drying are the factors that need to be addressed (Fukushima, 2000). Other workers have studied the functional properties of lima bean, mung bean, chickpea, winged bean and pigeon pea flours. (Adebowale and Lawal, 2004) reported a comparative study on the functional properties of bambarra groundnut, jack bean and mucuna bean flours. (Mueseset *et al.*, 1993) conducted experiments to investigate the possibility of processing pigeon pea to yield intermediate flour with good functional characteristics for food product development. Onimawo and Asugo, (2004) studied the effects of germination on the nutrient content and functional properties of pigeon pea flour.

2.3.1 Protein solubility

Fennema, (1996) reported of the functional properties of proteins, such as solubility and emulsifying activities, are in turn highly dependent on many factors, such as pH and the type and amount of salts present also he explained nitrogen solubility to be influenced by several solution condition, such as pH, ionic strength, temperature and the presence of organic solvents. The functional properties of proteins are often affected by protein solubility and those most affected are foaming, emulsification and gelation. The solubility of a protein is the thermodynamic manifestation of the equilibrium between protein protein and protein solvent interactions (Damodaran, 1996). The major interactions that influence the solubility characteristics of protein are hydrophobic and ionic in nature. Hydrophobic interactions promote protein-protein interactions and result in decreased solubility. Whereas ionic interactions promote protein-water interactions and result in increased solubility (Fennema, 1996). Jianmei Yu and Ipek, (2007) have explained that roasting of peanuts significantly decreases protein solubility in peanut flour in the pH range 3.5–10.0 compared to that in raw peanut flour. This decrease was attributed to the effect of heating which increased the surface hydrophobicity of protein due to unfolding of molecules and molecular size effects through hydrophobic interactions and disulfide formation. Nitrogen solubility for many legume seeds in aqueous solution of hydrochloric acid and sodium hydroxide at different pH values was studied by many investigators (Hang et al, 1970; Coffman and Garcia, 1977 and Thompson, 1977). Their results explained that the highest nitrogen solubility occurred at pH (1-2) in the acidic range and above pH 7 in the basic range with minimum solubility occurring in the pH range (4-5). Jianmei Yu and Ipek, (2007) reported that the minimum protein solubility was observed at pH 3.5-4.5 and maximum solubility at pH 10 or higher. Chickpea protein solubility was minimum at pH 4.3 (Sanchez-Vioqueet *al*, 1999).

2.3.2 Water retention capacity (WRC)

Water retention is defined as the ability of the food material to hold water against gravity (Hansen, 1978). The water retention capacity is the sum of bound, hydrodynamic and physically entrapped water (BeMiller and Whistles, 1996). Hydration or rehydration is the first and perhaps most critical step in imparting desirable functional properties to proteins in a food system. Water that interacts with the protein molecule exhibits different properties from those of “free” water. Water retention capacity directly related to the manner in which the protein interacts with water. The primary protein-water interaction is believed to take place at various water binding sites on the protein molecule. Theories that explain the mechanism of action of these different water binding sites are reviewed. Intrinsic factors affecting water-binding capacity of food proteins include amino acid composition, protein conformation and surface polarity/hydrophobicity (Barbut, 1999). However, food processing methods have important impacts on the protein conformation and hydrophobicity. With respect to water-holding capacity, the denatured proteins bind more water through exposure of hydrophilic groups (Kinsella, 1982). Jianmei Yu and Ipek, (2007), reported that, during roasting, peanut proteins were denatured by high temperature, exposing more hydrophobic sites, which explained the reduced water retention of peanut protein. Different protein structures and the presence of different hydrophilic carbohydrates might be responsible for variations in the WRC of the flours. Flours with high water absorption have more hydrophilic constituents, such as polysaccharides.

2.3.3 Fat absorption capacity (FAC)

Interactions of water and oil with proteins are very important in food systems because of their influence on the flavor and texture of foods. Variations in the presence of non-polar side chains,

which might bind the hydrocarbon side chains of oil among the flours, possibly explain difference in the oil binding capacity of the flours (Adebowale and Lawal, 2004). The ability of flours to absorb and retain water and oil may help to improve the binding of the structure, to enhance flavor retention, to improve mouth feel and reduce moisture and fat losses of extended meat products (McWatters and Heaton, 1979). Hutton and Campbell, (1981) explained the role of protein-lipid interactions, in the absorption capacity of protein. They suggested that, no covalent bonds, such as hydrophobic, electrostatic and hydrogen bonding significantly contributes in stabilization of protein-lipid complexes. Therefore surface hydrophobicity was suggested to be the major determinant of fat binding capacity (Voutsinas and Nakai, 1983).

2.3.4 Bulk density (BD)

Bulk density is a physiochemical property, depends on interrelated factors including intensity of attractive, interparticle factors, particle size, number of contact points (Peleg and Bagley, 1983). It also depends on type of solvent used to extract the protein products (Wang and Kinsella, 1976), and on method of drying (Bryant, 1988). The bulk density of field pea flour was found to be (0.541–0.562 g/ml), while the bulk density of pigeon pea flour was (0.471–0.467 g/ml) (Kaur et al, 2006). The Bulk densities of 0.536–0.571 g/ml in chickpea flours (Kaur and Singh, 2005). Higher BD is desirable since it helps to reduce the paste thickness, which is an important factor in convalescent and child feeding (Padmashree et al, 1987).

2.3.5 Gelation

Interactions between protein molecules when induced by heating may give rise to protein aggregates. When such heat- induced aggregation takes place at a protein concentration high enough to entrap water in the three-dimensional matrices of the aggregates, gelation or

coagulation will result (Kim et al, 1990). Gelatinization, the process by which the internal structure of the granule disintegrates, releasing polysaccharide into the surrounding medium is accompanied by a 12 variety of changes. When starch variety of changes. When starch granules are heated in water beyond a critical temperature, the granules absorb a large amount of water and swell to many times their original size. Over a critical temperature range, the starch granules undergo an irreversible process, which is marked by crystalline melting and starch solubilization (Singh *et al.*, 2005). Least gelation concentration (LGC) for various legume flours ranged from 12% to 14%. The lower the LGC, the better is the gelating ability of the protein ingredient (Akintayoet *al.*, 1999). On the other hand, sorghum flour concentrations for gelation were significantly lower than those of partially defatted peanut flour. Sorghum flour contains starch, which induced gelation due to starch and/or starch-protein interactions. Partially defatted sorghum-peanut composite flour required a higher flour concentration than sorghum flour for gelation because the starch content decreased due to fortification with peanut flour (Singh and Singh, 1991).

2.3.6 Foaming properties

The foaming property of a protein refers to its ability to form a thin tenacious film at gas-liquid interfaces so that large quantities of gas bubbles can be incorporated and stabilized (Fennema, 1996). The formation of foam is analogous to the formation of emulsion. In the case of foam, water molecules surround air droplets, and air is the non-polar phase. The amphipathic character of proteins makes them good foaming agents that work at air-water interfaces to prevent bubble coalescence. Soybean proteins are extensively used in the preparation of aerating or whipping agents; however, many such preparations that show excellent foam expansion are of little practical value because of instability. Thermal instability of many oilseed proteins limits

their applications. The factors that affecting foaming formation and stability are pH, sugars, lipids and protein concentration (Fennema, 1996).

2.4.6.1 Foaming capacity (FC) and foaming stability (FS)

The foaming capacity of a protein refers to the amount of interfacial area that can be created by the protein and foam stability refers to the ability of protein to stabilize against gravitational and mechanical stresses (Fennema, 1996). The foams produced by legume flours were relatively thick with low foam volume but high foaming stability. Foaming stability (3% w/v dispersion) for legume flours, it was determined by measuring the decrease in volume of foam as a function of time. Foam volume changes, as a function of time, for legume flours. Instability of foams is indicated by drainage of liquid from the lamellae and by an increase and then rupture in the size of bubbles (Satheet *al.*, 1982a).

2.3.7 Emulsifying properties:

Emulsification of proteins is closely related to the conformation of proteins and interaction of adsorbed molecules at the oil/water interface. Emulsion formation relies on a fast adsorption unfolding and reorientation (Damodaran, 1996). Factors that affect emulsifying properties are adsorption kinetics, interfacial load, decrease of interfacial tension, rheology of the interfacial film and surface hydrophobicity of the interfacial film (Das and Kinsella, 1990). The presence of NaCl improved the emulsifying properties of cowpea, fenugreek and sesame proteins (El Nasri and El Tinay, 2007; Inyang and Iduh, 1996; Ragab et al 2004). On the other hand, addition of CaCl₂ prior to emulsification increased the average droplet diameter and reduced creaming stability of the emulsion (Ye and Singh, 2000).

2.3.7.1 Emulsion capacity (EC)

Emulsion capacity is the volume (ml) of oil that can be emulsified per gram of protein before phase inversion occurs (Fennema, 1996). The capacity of proteins to unfold at an interface depends on the conformational stability of their tertiary structure. Soy protein isolate and soy protein concentrate vary in their emulsification capacities (Hutton and Campbell, 1977a, 1977b) according to their composition and the stress to which they have been subjected.

2.3.7.2 Emulsion activity (EA) and emulsion stability (ES)

Some studies have suggested that the good emulsifying activity of a protein is related to its high solubility (Inyang and Iduh, 1996; El Nasri et al., 2007; Ragab et al., 2004). The type of protein and method of preparation affect the formation and stability of emulsion. The emulsion stability which reflects the ability of proteins to impart strength to emulsion for resistance to stresses (Patel and Kilara, 1990).

2.3.8 Dispersibility

Ease of dispersibility is important in food formulation. The dispersibility of a mixture in water indicates its reconstitutability. (Kullarni *et al.*, 1991). The better temperature, ionic composition, pH and degree of agitation of the solvent are major factors affecting dispersibility (Kinsella, 1976). The dispersibility of sesame protein isolate was significantly higher at neutral and alkaline pH than acidic pH (Khalid *et al.*, 2003). The dispersibility of raw chickpea flour was 25.3% (Milancarrillo et al, 2000).

2.3.9 Wettability

Ease of wettability is important in food formulation. Wettability of proteins is affected by surface polarity, topography, texture, area and by the size and microstructure of the protein particles (Hagerdal and Lofqvist, 1978).

2.4 Utilizations of groundnuts:

The groundnut is perhaps the world's most widely studied food protein oilseed. Advantages over other oilseeds include relatively bland flavor, minor color problems, and minimal preparation requirements. Products in use throughout the world include boiled groundnut, roasted full-fat or partially defatted groundnut, groundnut butters, grits and flours (full-fat or defatted), defatted groundnut, protein concentrates, and protein isolates. Compounded food applications include fortified breads and bakery products, snacks, meat products, extended milks, cheese and curd type products, and various mass-feeding foods in developing countries. The pattern of utilization of groundnut differs among the developed and the developing countries (Freeman *et al.*, 1999). They are used for human nutrition in developing countries to supplement cereals, beverages and skim milk (Natarajan, 1980; Rustomet *al.*, 1996). In the United States (US), the major proportion of produce is processed for direct consumption as groundnut butter, salted groundnut and confectionery, whereas in Asian countries, particularly in India, groundnut is utilized primarily as an oilseed and as such the bulk of the produce is directed for crushing for expulsion of oil (Carley and CaCl₂ prior to emulsification increased the average droplet diameter and reduced creaming stability of the emulsion (Ye and Singh, 2000).

2.4. Groundnut sauce (Ose Oji)

Groundnut sauce is a sauce made from groundnut, often with various other ingredients. It is a staple of African cuisine but is also eaten in East Asia (Taiwan), the United States (mainly in Virginia) and other areas around the world. It is also common in various Latin American regions, such as Argentina, Bolivia and Peru, where it can sometimes be served with bone meat and hollow short pasta or fries. In Ghana it is often eaten with fufu or omotuo. Groundnut sauce is also a native soup of the Benin (Edo) people in Nigeria and it is often eaten with pounded yam. Some of the essential ingredients used in making it are *Piper guineense* (uziza seed) and *Vernonia amygdalina* (bitter leaf), It is popularly known by Nigerians in an Igbo dialect language as 'Oseoji'

It is a delicacy that is prepared from groundnut which is mashed into a paste, usually termed as groundnut paste. Groundnut sauce is eaten with fufu, banku, kenkey and so on. It is a delicacy that Ghanaian and people in other African countries consume, such as in Sierra Leone. It is popularly known by Ghanaians in an Akan language called *Nkatenkwan*.

2.5. Food Borne Illnesses

Food borne illnesses are globally important, as they result in considerable morbidity, mortality, and economic costs. Food borne illnesses (also called food poisoning) are infections or irritations of the gastrointestinal (GI) tract caused by eating contaminated food or beverages (Mahoney *et al.*, 2007). Infectious organisms including bacteria, viruses and parasites or their toxins are the most common causes of food poisoning. Infectious organisms or their toxins can contaminate food at any point of processing, production, growing, harvesting, storing, shipping or preparing (Biswaset *al.*, 2010). Natural and manufactured chemicals in food products

also can make people sick. The potential for the contamination of street foods with pathogenic microorganisms has been well documented and several disease outbreaks have been traced to consumption of contaminated street foods. There are more than 250 known food borne diseases (Feglo and Sakyi, 2012). Many microbes can spread in more than one way, so it may not be immediately evident that a disease is food borne. The distinction matters should be identified because public health authorities need to know how a particular disease is spreading and the appropriate steps should be taken to stop it (Snoeyenbos *et al.*, 1969).

2.5.1. Risk factors contributing to food borne infection

- a) **Older adults:** As anyone gets older, immune system may not respond as quickly and as effectively to infectious organisms as when younger (Oladipo and Adejumobi, 2010).
- b) **Pregnant women:** During pregnancy, changes in metabolism and circulation may increase the risk of food poisoning. The reaction may be more severe during pregnancy. Rarely, the baby may get sick, too (Oladipo and Adejumobi, 2010).
- c) **Infants and young children:** Their immune systems haven't fully developed. People *with chronic disease:* Having a chronic condition such as diabetes, liver disease or AIDS or receiving chemotherapy or radiation therapy for cancer reduces immune response (Oladipo and Adejumobi, 2010).

2.5.2. Causes of Food Borne Illnesses

Although a number of different infectious pathogen may be contracted from foods under certain circumstances, there are those that are contracted exclusively or predominantly from the consumption of food products. The recognized food borne pathogens include multicellular animal parasites, protozoa, fungi, bacteria, viruses, and possibly prions. Some harmful microorganisms may already be present in foods when they are purchased.

2.5.3. Major sources contributing to microbial contamination of food

2.5.3.1. Vending Location: Food Handling and Waste Disposal

The conditions under which some street vendors operate are reported to be unsuitable for the preparation and selling of food. The two major sources from where the contaminants can enter the preparation area are: Improper food handling and waste disposal.

2.5.3.2. Food Handling

Unsanitary handling of street foods by the some of the vendor has been commonly found to be the source of contamination. The vendors can be carriers of pathogens like *Escherichia coli*, *Salmonella*, *Shigella*, *Campylobacter* and *S. aureus* who eventually transfer these food borne hazards to the consumers. The hands of the food handlers are the most important vehicle for the transfer of organisms from faeces, nose, and skin to the food (Rane, 2011).

2.5.3.3. Waste Disposal

Few vendors congregate in overcrowded areas where there are high numbers of potential customers, which usually provide limited access to basic sanitary facilities. Hence, the contamination of street foods is often linked to the waste generated by food processing, that is usually dumped near the vending site. The lack of facilities for liquid drainage and wastewater and garbage disposal encourages wastes to be thrown into nearby streets and gutters. Such areas act as habitats for rodents, breeding points for flies and media for growth of microorganisms (Rane, 2011).

2.3.3.4. Quality of Raw Materials: Water and Other Material

The quality of raw materials used in the preparation of street foods is very important as their contamination can persist through preparation and or cooking.

2.3.3.5. Utensils and Equipment

Chemical and Microbial Contaminants Use of proper utensils for cooking and storage of prepared food is often critical to the safety of street vended foods. Poor quality of material coupled with improper practices may lead to toxin formation, pathogen growth or recontamination.

2.3.3.6. Food Preparation: Storage

An important issue influencing food contamination and contributing to further increase in contamination is food storage temperature. The preparation of food long before its consumption, storage at ambient temperature, inadequate cooling and reheating, contaminated processed food and under cooking are identified as the key factors.

2.3.3.7. Storage

Foods are often held for several hours after cooking and this includes overnight holding at ambient temperatures, until sold, and thus can harbor high microbial populations. Besides, some of the foods are held in the pans in which they are cooked, until sold or reheated, which results in longer holding time, hence creating favorable conditions for the growth of food borne pathogens (Rane, 2011).

2.3.3.8 Personal Hygiene of the Vendors or Food Handlers

According to World Health Organization, food handling personnel play an important role in ensuring food safety throughout the chain of food production, processing, storage and preparation. Mishandling and disregard of hygienic measures on the part of the food vendors may enable pathogens to come into contact with food and in some cases to survive and multiply in sufficient numbers to cause illness in the consumer. Some food handlers may introduce biological hazards by cross contamination after handling raw materials when they suffer from specific diseases and physical hazards by careless food handling practices. Most of the vendors pack the food in polythene bags for their customers. When packing these foods, they blow air into the polythene bags to open them, in this process a number of pathogens can be passed on to the consumer (Ohiokpehai, 2003).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Sample Collection

Ten (10) different types of ose-orji were collected from the restaurants of five (5) different local governments in Benin (Ovia north east local government, Oredo local government, Egor local government, Uhumwonde local government, Ikpoba Okha local government) to perform cultural fungi analysis of microbial flora. The samples were collected on February 1st. All the Ice creams were carried using a different sterile nylon bag and transported to the lab. The purchasing time was in between 10am to 5pm.

3.2. Materials and methodology

The materials used for the work were autoclave, refrigerator, hot air oven, malt extract agar (MEA) and potato dextrose agar (PDA), antibiotic, Bunsen burner, Test tubes and rack, Cotton wool, ethanol, Beaker, Conical flask, Petridishes, Pipettes and tips, and Aluminium Foil.

3.3 Reagents

Distilled water, Ethanol.

3.4 Sterilization of Glass Wares

Petri-dishes, test tubes, conical flasks, beakers, pipettes, etc. was sterilized in hot air oven at 180°C for two hours (2 h) and stored at 40C.

3.5 Preparation of Culture Media

Pour plate techniques were performed for fungi isolation. Potato Dextrose Agar (PDA) & Malt Extract Agar (MEA) were used and both of them were prepared according to the instructions provided by the manufacturer company.

Potato Dextrose Agar was weighed at 39g and Malt Extract Agar was weighed at 15g which was added in 1000ml of distilled water which was properly dissolved using water bath and was autoclaved at 121⁰ C for 15minutes. The prepared medium was allowed to cool for few minutes then poured in 10 different sterile petri dishes and allowed to solidify within 30minutes on a sterile table. The table was sterilized using 70 percent of ethanol and cotton wool to clean the surface of the table.

3.6. Preparation of Sample:

The microbiological test was performed in Mycofarm research laboratory. 1ml of ose-orji sample was put into 9ml of sterile water in a test tube and the mixture was shaken thoroughly to mix it well to get tenfold (10^3) dilution. The dilution was then labeled as A1. One (1) ml of the diluted sample before settling was removed from the suspension using a sterile pipette and transferred in to a sterile test tube containing 9mls of sterile water. It was thoroughly mixed again and labels A2, and transferred into third labeled A3 where 0.1 ml was plated on the petridish.

3.7. Making pour plate for fungi culture

Ten prepared sterile plates were labeled using five plates per agar media. Exactly 0.1ml was pipetted out from each of the labeled dilution using a micro pipette unto each plate as

labeled by its diluting factor which is 10^{-1} , 10^{-2} , 10^{-3} , and 1ml of antibiotics was pipette to each petri dish to inhibit the growth of bacteria. Potato dextrose agar (PDA) was then added to 5 plates and malt extract agar was added to the remaining 5 plates. The plates were swirled so that the inoculums will mix with the agar. The plate was opened quickly to avoid contamination. The plates (10) were placed invertedly at room temperature for 24-72 hours for observation of growth.\

$$\text{Total fungi count (cfu/mL)} = \frac{\text{Number of colonies on plates} \times 1 \times \text{Dilution factor}}{\text{volume of aliquot}}$$

3.8. Isolation pure culture

Using agar slant, individual fungal colony from any of the plates representing each colony was collected using a sterile wire loop, as taken from a high diluted plate as its tends to have pure colonies that are well separated. The wire loop was sterilized, then the selected agar plate was quickly opened near the Bunsen burner, the selected colony was gently picked and the slant in the bjou bottle was opened as the tip of the bottle was flamed. The sterile wire loop with the colony was smeared at the surface of the sterile agar slant. This procedure was repeated for different growth observed macroscopically. The slant tip was re-flamed, covered and kept at room temperature for 3 to 7days. This was done to obtain pure colony.

3.9. Characterization and identification of isolates

The growth pattern, pigmentation and size of colonies were recorded to aid in the identification of the organisms. Fungal colonies were identified as described by Harrigan (1984). cultural characteristics. The fungal cultures were identified on the basis of macroscopic (colonial morphology, color, shape, diameter, appearance of colony and basis of Lactophenol staining) and microscopic (septation in mycelium, presence of specific reproductive structures).

3.10. Statistical analysis

Differences between means were evaluated by Duncan's test. A significance difference was established at $p = 0.05$

CHAPTER FOUR

RESULTS

Table 4.1 shows the mean difference of *ose oji* samples across the days analyzed. The result revealed that the Mean cfu/ ml of colonies counted on malt extract agar for *ose-orji* samples collected at different locations ranged from $0.20 \pm 4.05 \times 10^3$ cfu/ml. The result of the statistical analysis for table 1 showed that there was significant difference ($p > 0.05$) between total heterotrophic count of samples collected from different locations and there was a significant difference between *ose oji* sampled at different day.

Figure 4.1. Shows the trend in mean total heterotrophic count for *ose oji* samples from day 0- day 4. The results revealed the microbial increase from day 0-day 4.

Table 4.2. Shows the mean difference of *ose oji* samples across the days analyzed. The result revealed that the Mean cfu/ ml of colonies counted on potato dextrose agar for *ose oji* samples collected at different locations ranged from $0.50 \pm 4.90 \times 10^3$ cfu/ml. The result of the statistical analysis for table 1 showed that there was significant difference ($p > 0.05$) between total heterotrophic count of samples collected from different locations and there was a significant difference between *ose oji* sampled at different day.

Figure 4.2. Shows the trend in mean total heterotrophic count on potato dextrose agar for *ose oji* samples from day 0- day 4. The results revealed the microbial increase from day 0-day 4.

Table 4.1: Total heterotrophic fungi count (THFC) (CFU/mL x 10³) on malt extract agar (MEA) from day 0- day 4

SAMPLES	(MEAN ± STD)				
	DAY 0	DAY1	DAY 2	DAY3	DAY4
A	1.55± 0.21	2.10±0.7	2.60 ± 0.14	3.25 ± 0.05	4.05 ± 0.07
B	1.10 ± 0.02	2.20±1.4	2.80 ± 0.14	4.00 ± 0.04	6.51 ± 0.21
C	0.20 ± 0.04	2.6±0.03	3.70 ± 0.14	3.90 ± 0.04	3.75 ± 0.05
D	1.10 ± 0.02	1.70±0.10	2.35± 0.01	3.10 ± 0.04	3.90 ± 0.02
E	1.05 ± 0.05	1.40±0.10	2.95 ± 0.02	3.05 ± 0.07	3.70 ± 0.04

P- value within row > 0.05

P- value btw columns > 0.05

Key:

Sample A= Ovia north east local government, sample B= Oredo local government, sample C= Egor local government, sample D= Uhunmwonde local government, sample E= Ikpoba Okha local government.

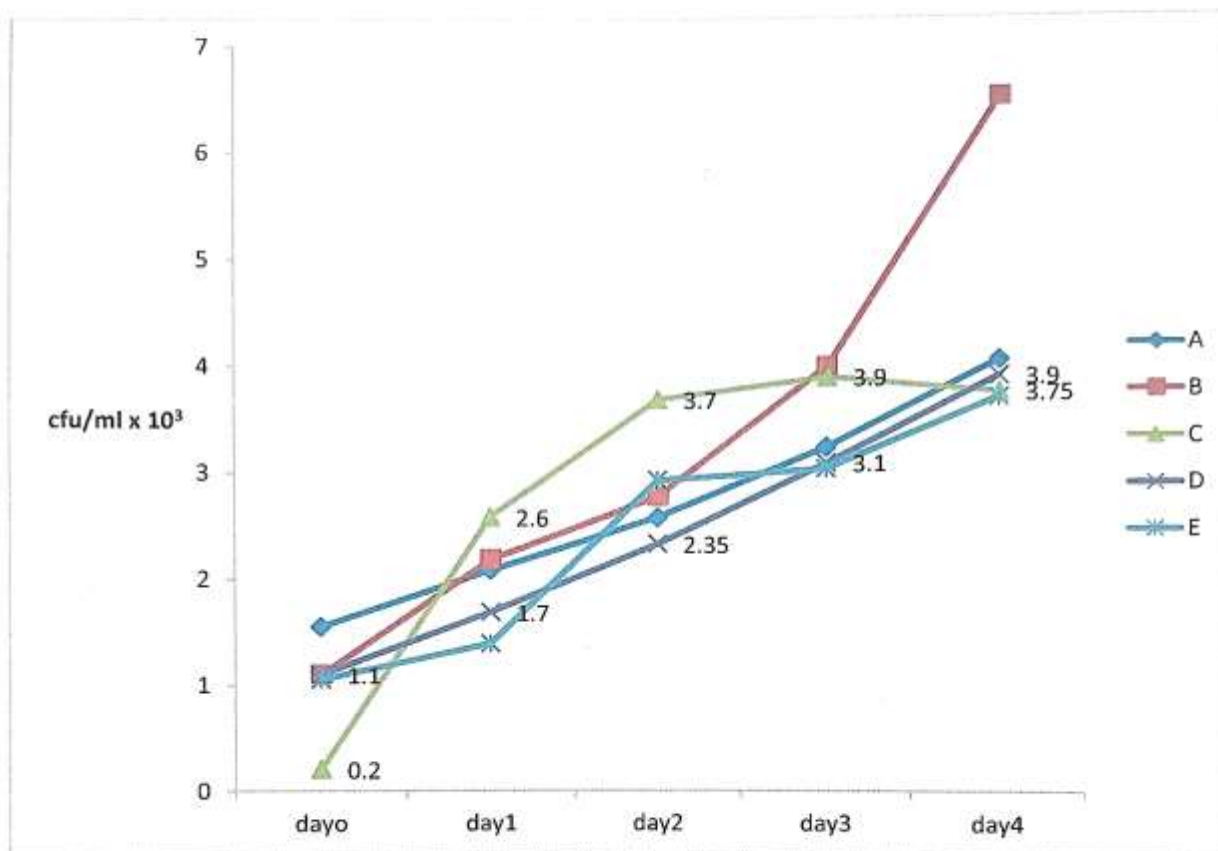


Figure 4.1: Total heterotrophic fungi count (THFC) (CFU/mL x 10³) on malt extract agar (MEA) from day 0- day 4

Key :Sample A= Ovia north east local government, sample B= Oredo local government, sample C= Egor local government, sample D= Uhumwonde local government, sample E= Ikpoba Okha local government.

Table 4.2: Total heterotrophic fungi count (THFC) (CFU/mL x 10³) on potato dextrose agar from day 0 – day 4

Samples	(MEAN ± STD)				
	DAY 0	DAY1	DAY 2	DAY3	DAY4
A	0.50± 0.10	2.95 ± 0.07	3.05 ± 0.07	3.60 ± 0.04	4.80 ± 0.27
B	3.00±1.20	3.70 ± 0.30	4.20 ± 0.28	4.60 ± 0.30	5.20 ± 0.28
C	1.80±1.40	2.40 ± 0.42	3.20 ± 0.14	3.05 ± 0.07	4.90 ± 0.14
D	0.70±1.40	2.60 ± 0.14	3.85 ± 0.07	4.30 ± 0.28	4.85 ± 2.07
E	2.10±0.70	2.80 ±0.14	2.90 ± 0.14	3.20 ± 0.14	3.90 ± 0.14

P- value within row > 0.05

P- value btw columns > 0.05

Key: sample A= Ovia north east local government, sample B= Oredo local government, sample C= Egor local government, sample D= Ujunmwonde local government, sample E= Ikpoba Okha local government.

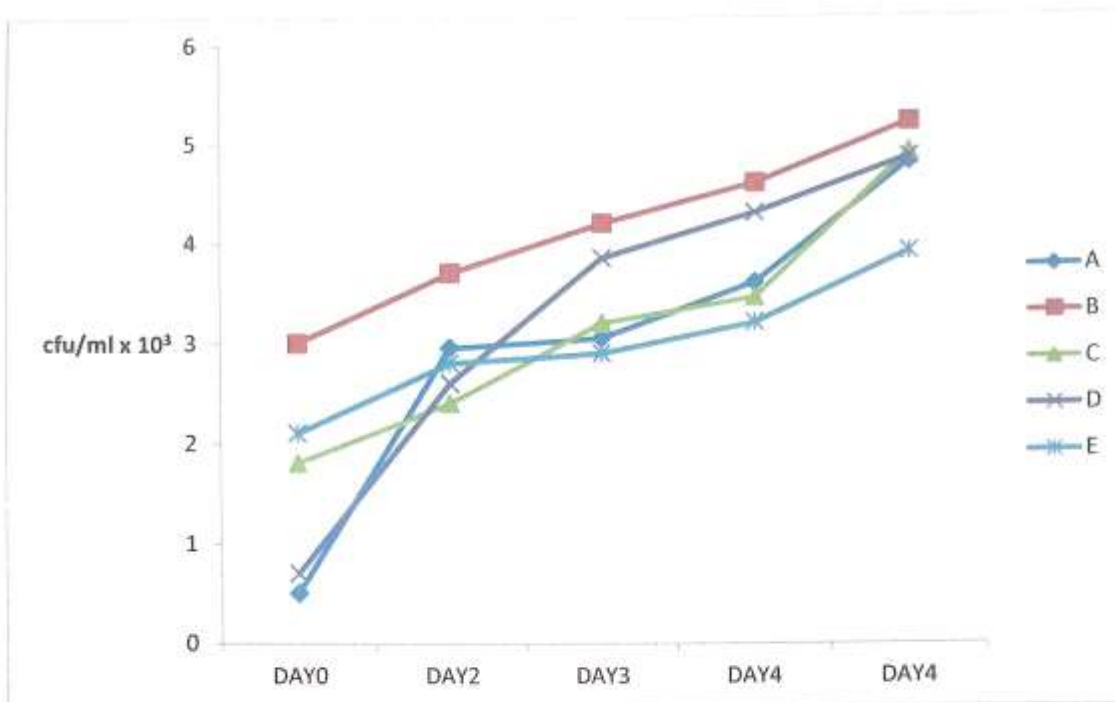


FIGURE 4.2: Total heterotrophic fungi count (THFC) (CFU/mL x 10³) on potato dextrose agar from day 0 – day 4

Key: sample A= Ovia north east local government, sample B= Oredo local government, sample C= Egor local government, sample D= Uhumwonde local government, sampleE= Ikpoba Okha local government.

Table 4.3 shows the cultural and microscopic characteristics of the fungal isolates. The fungi isolated from *ose-oji* samples from the various markets as shown in table were *Penicillium chrysogenum*, *Aspergillus niger*; *Mucor mucedo*, *Aspergillus flavus* and *Rhizopus* spp. The isolates were identified based on their cultural and microscopic characteristics as described in the same table.

Table 4.4 and figure 3 shows the percentage occurrence of fungi isolates. A total of 5 fungi isolates were identified. The table shows that *Penicillium chrysogenum*. (26.31 %) and *Mucor mucedo* (26.31%) as the most predominant isolates.

Table 4.3: Cultural and microscopic characteristics of the fungal isolates from groundnut sauce

Characteristics	F1	F2	F3	F4	F5
Cultural	Green Colony with Reverse side Dirty white	Black fluffy Colony with Reverse side Yellow	White flat Colony with Reverse side Cream	Green-yellow Colony with reverse side yellow	White fluffy Colony with reverse side cream
Microscopic					
Nature of Septate	Septate	Septate	Non-septate	Septate	Non-septate
Colour of spore	Green	Brown	Cream	Green	cream
Types of spore	Conidiophores	Conidiophores	Sporangiophor	Conidiophores	Sporangiophore
Appearance	Brush-like	Foot cells	sporangium	Foot cell	Rhizoid
Of special	Conidia				
Structure					
Possible Isolate	<i>Penicillium chrysogenum</i>	<i>Aspergillusniger</i>	<i>Mucormucedo.</i>	<i>Aspergillus Flavus</i>	<i>Rhizopus</i> spp.

Table 4.4; Occurrence of fungal isolates in groundnut sauce

Fungal isolates	Occurrence (%)
<i>Penicillium italicum</i>	26.31
<i>Aspergillusniger</i>	15.70
<i>Mucormucedo</i>	26.31
<i>Aspergillus Flavus</i>	15.70
<i>Rhizopusspp.</i>	15.70

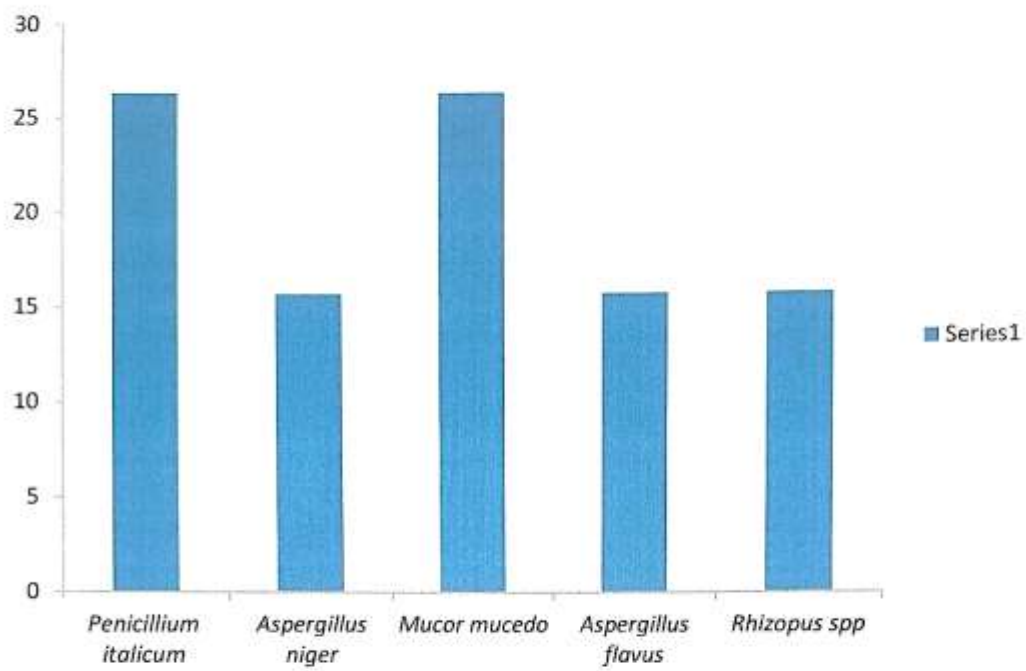


Figure 4.3: Bar graph showing percentage occurrence of fungal isolate

CHAPTER FIVE

DISCUSSION

The main objective of this study was to determine the fungi contaminants of groundnut sauce (*OSE-OJI*) sold in Benin City. The Total heterotrophic bacterial count (THBC) was used as important indicator of the microbial quality of *ose-oji* samples. A total of 10 samples from five vendors (in triplicate) were cultured for total heterotrophicfungi counts on two different fungi media. It was observed that the mean fungi count of the samples ranged from $0.20 \pm 4.05 \times 10^3$ cfu/ml on malt extract agar and $0.50 \pm 4.90 \times 10^3$ cfu/ml potato dextrose agar on agar. The result of the statistical analysis for table 1 and 2 showed that there was significant difference between total heterotrophic count of samples collected from different locations and using different media for heterotrophic fungi count. The mean THFC obtained was lower than that reported by Ogodo *et al.* (2018) (1.94×10^7 cfu/g to 2.44×10^7 cfu/g) and Yabaya *et al.* (2012) (2.0×10^7 cfu/g to 2.23×10^8 cfu/g). Also, the result of the present study is in line with the report, which reported fungi load of 3.2×10^3 cfu/ml to 4.7×10^3 cfu/ml in peanut sauce (Adebesinet *et al.*, 2001).

The morphological, cultural and biological characteristics of microbial isolates revealed the following fungi genera; *Penicillium chrysogenum*, *Aspergillus niger*, *Mucor mucedo*, *Aspergillus Flavus* and *Rhizopus* spp. Other studies in Kenya, Benin and Mali, have also revealed the occurrence of *Aspergillus*, *Fusarium* and *Penicillium* and other groundnut products (Adjou *et al.*, 2012; Keita *et al.*, 2013; Ndung'u *et al.*, 2013). The presence of *Aspergillus* sp. implies a risk of mycotoxin production and represents a health risk for the consumers (Sultan and Magan, 2010). According to Pittet (1993), the mycotoxins produced by *Aspergillus* spp. include aflatoxins and ochratoxin A (OTA). Mycotoxins have attracted worldwide

attention due to the significant losses associated with their impact on human and animal health, and consequent national economic implications (Bhat and Vashanti, 1999). Mycotoxins can be acutely or chronically toxic or both depending on the nature of toxins and the dose consumed. In human, acute diseases include liver and kidney damage, attack on central nervous system (CNS), skin diseases and hormonal effects. Among the mycotoxins, aflatoxins produced by *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nomius* are natural carcinogenic compound causing mutation (Deng and Ma, 1998).

The observed fungi contamination of the studied ose- oji (groundnut sauce) exposing the consumers to a potential risk of acquiring food borne disease. Indeed, food borne illnesses of microbial origin are a major international health problem associated to food safety in developing countries (WHO, 2002). The high susceptibility of groundnuts contamination is mainly due to their nutritional content, useful to numerous fungi. Contamination of street-vended food such as groundnut sauce has been attributed to exposure to polluted environment, poor sanitation and poor hygienic practices by the vendors (Mensah *et al.*, 2002). There have been several suggested interventions to improve the hygiene of street foods such as groundnut sauce, which includes (1) education and training programs for vendors, (2) the improvement of vendors' equipment for preparation and storage, (3) the provision of adequate sanitation and the adoption of HACCP system in order to improve the efficiency of the surveillance system by detecting the hazards and focusing on the critical control points (WHO, 2002).

5.1. CONCLUSION

The studied groundnut sauce sold in Benin City is nutritive and could meet the dietary needs of the population when consumed with other foods. Indeed, this sauce constitute a valuable sources of protein, fat, crude fibre and minerals. However, the presence of toxigenic fungi as *Aspergillus flavus* and other fungi species presents highlight a potential public health problem concerning the consumption by the consumers. Therefore, the need to educate both sellers and consumers on processing, food handling procedures and personal hygiene would help to minimize fungal contamination in order to obtain good sanitary groundnut sauce.

APPENDIX I

STATISTICAL ANALYSIS OF TABLE 1

Anova: Two-Factor Without Replication

<i>SUMMARY</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>
Row 1	2	50000	25000	0
Row 2	2	100000	50000	2E+08
Row 3	2	115000	57500	1.01E+09
Row 4	2	55000	27500	3.13E+08
Row 5	2	85000	42500	3.13E+08
Column 1	5	240000	48000	4.33E+08
Column 2	5	165000	33000	2.83E+08

ANOVA

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Rows	1.59E+09	4	3.96E+08	1.243137	0.419025	6.388233
Columns	5.63E+08	1	5.63E+08	1.764706	0.254761	7.708647
Error	1.28E+09	4	3.19E+08			
Total	3.42E+09	9				

STATISTICAL ANALYSIS FOR TABLE 2

Anova: Two-Factor Without Replication

<i>SUMMARY</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>
Row 1	2	405000	202500	1.71E+10
Row 2	2	630000	315000	6.05E+09
Row 3	2	410000	205000	8.45E+09
Row 4	2	495000	247500	2.53E+10
Row 5	2	475000	237500	3.61E+09
Column 1	5	970000	194000	5.17E+09
Column 2	5	1445000	289000	8.48E+09

ANOVA

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Rows	1.66E+10	4	4.15E+09	0.437525	0.778484	6.388233
Columns	2.26E+10	1	2.26E+10	2.376564	0.198025	7.708647
Error	3.8E+10	4	9.49E+09			
Total	7.72E+10	9				

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