FUNGI ASSOCIATED WITH MAIZE AND PAP VENDED IN NEW BENIN

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BY

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CERTIFICATION

This is to certify that this research project work was done by **Christabel Izegboya OJEISEMI (Miss)** in the department of Microbiology, Faculty of Life Sciences, University of Benin, Benin city, under my supervision.

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APPROVAL

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

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

DEDICATION

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

ACKNOWLEDGEMENT

I want to first appreciate God for His mercies and grace that has brought me this far, for helping me realize many of my dreams within this great citadel of learning. To Him be all the glory and adoration.

To my supervisor, Mr. B. O. OMOREGIE, for being very patient, for his guidance, advice and corrections I say thank you. May the Almighty continue to bless and provide the best for you and your family. To all my lecturers in the Department 0f Microbiology I say thank you.

My sincere appreciation and gratitude goes to my lovely parents, Mr. and Mrs. Ojeisemi for their unending love, financial support and believing in me. I pray God gives them long life to see me and my siblings achieve our dreams in life and enjoy the fruits of their labour. To my siblings, I love you all so much.

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ABSTRACT

Maize (*Zea mays*) is a cereal crop which is an important raw material in human diet. It is an annual grass in the family *Poaceae* and is a staple food crop grown all over the world. The aim of this study was to isolate and identify fungi from maize and its products (pap). A total of 6 samples from six vendors were cultured for total heterotrophic fungi counts on different potato dextrose agar (PDA). The total fungi count of maize and pap samples ranged from $0.4 \times 10^6 – 1.4 \times 10^6$ cfu/g and 1.40×10^2 - 2.00×10^6 cfu/g respectively. A total of 37 fungal isolates were identified. The morphological, cultural and biological characteristics of fungi isolates revealed the following fungi species; *Penicillium oxalium, Penicillium italicum, Aspergillus niger, Aspergillus flavus, Mucor mucedo, Penicillium oxalium, Cladiosporium* sp., *Saccharomyces* spp., *Mucor* sp., *Clavispora* spp., Cryptomonas spp. and *Saccharomycodes, Galcatomyces*. The results of fungi diversity in different samples revealed that samples A (21.6 %), C (24.3 %) as the most contaminated isolates, while sample D (10.8) and F (10.8) was the least contaminated. This study revealed the common fungi flora of maize and pap samples sold in local markets in Benin City. This study revealed the common fungi flora, their distribution in maize and pap and some possibly fungi contaminants which may not be directly associated with maize and pap. The distribution of fungi species in maize and pap samples may be dependent upon environmental conditions at the time of harvest, processing and the difference between the storage structures. It is therefore recommended that further studies be undertaken to understand the role of each fungi general isolated in this study and their source of contamination.

CHAPTER ONE

INTRODUCTION

Maize (*Zea mays*) is a cereal crop which is an important raw material in human diet (Adebolu *et al.,* 2007). It is an annual grass in the family *Poaceae* and is a staple food crop grown all over the world. It is believed that maize originated from Mexico and Central America. Corn grows in ears, each of which is covered in rows of kernel that are protected by the silk like threads called corn silk. Corn is scientifically known as *Zea mays*. Though corn is usually associated with yellow color, it actually comes in host of different varieties featuring an array of different colors. There are red, pink, purple and blue corns (Adhikari *et al*., 1994).

Maize is the most important raw material used in food production. The contamination of the raw materials by mould and mycotoxin are very frequent. This contamination could lead to nutrient losses and detrimental effect on animals and production. Drought, humidity, temperature, insect, infestation and rough handling have been suggested as factors which contribute to the presence of fungi and subsequently toxins in agricultural products (Aminigo and Akingbola, 2004). Fungal spoilage of corn reduces the nutritional value and palatability of the feed, thereby increasing its allergic potential and may result in mycotoxic contamination (Banigo *et al.*, 2002).

Fermented maize starch commonly known as "pap" is a fermented maize product obtained as smooth gel or mixed with boiling water to form a porridge, which has a sour taste. It is known as "*Ogi*" in the western part of Nigeria by the Yoruba's or *Akamu* in the Eastern or "*Akassa*" in the North by the Igbo's and Hausas respectively (Banigo *et al.*, 2002). Similar maize preparations are referred to as "*Akana*" and "*Kenkey*" in Ghana. It is a popular staple and most popular traditional weaning food in West African countries (Jaris, 2001). It is used

as weaning food by low income earners who cannot afford the more expensive imported weaning foods (Jay, 2005). Pap is mostly prepared using traditional fermenting and malting technologies which are simple but do not guarantee quality and lack of contaminations as well as lack of the appropriate nutritive value (Jay, 2005). It is prepared by soaking (steeping) in water for two to five days, grinding it (wet milling) and sieved to remove the husk. The main reason for fermenting maize grains is to convert starch contents in the cereals such that it does not require dilution. The fermenting process also removes the pathogens. Pap provides about 20-26 kcal/kg per day to an infant who has an average density of 0.26 kcal/kg (Kunele, 1999).

In most parts of Africa especially in Nigeria, children are fed with mashed adult foods. These foods are bulky and can cause malnutrition. The developments of nutritionally balanced calorie as dense weaning foods lead to the fermentation of maize to provide pap. The food must also be of the right quantity to satisfy the infant at one feeding. It is also a meal of choice for patients in need of soft and easily digestible foods (Jay, 2005). They are important energy food rich in carbohydrate with traces of vitamins, proteins and minerals and are natural antioxidants (Odunfa and Adeyele, 2000). Its reputation as the most popular traditional weaning food and its consumption by convalescent in the West African regions calls for a safe product, free of pathogen and any potentially hazardous microorganisms. The traditional fermentation processes of pap are usually spontaneous and uncontrolled (Odunfa, 1985) and have led to the loss of nutrients. The nutritive quality of maize porridge is very low resulting from low quality maize proteins and substantial loss of nutrients at the different stages of production (Omume and Adeosun, 2010).

The Microbiology of maize and its related products has been studied (Odunfa and Adeyele, 1985). New attention is presently on the use of starter cultures, which is solving numerous problems associated with the product capable of prevention and treatment of many

water borne disease using bacteriogenic lactic acid bacteria (LAB) (Osungbaro, 2009). Ozoh and Kuyanbana, (2006) increased the shelf-life of "pap" using a bacteriocin producing *lactobacillus* isolate. Pap is fairly acidic (pH 4.8), which tends to inhibit the growth of some organisms.

1.1 Aim and Objectives

Aim

The aim of this study was to identify fungi from maize seeds and papa

The specific objectives were to;

I. isolate, identify and characterize fungi present in maize seeds and its product pap,
II. determine the total heterotrophic fungal count in the maize seeds and pap,
III. determine the frequency and percentage frequency of occurrence of fungi isolates, and
IV. determine the biodiversity and percentage diversity of fungal isolates.

CHAPTER TWO

LITERATURE REVIEW

2.1. Maize

Maize (*Zea Mays L., Poaceae*) is the most important cereal in the world after wheat and rice, with regard to cultivation areas and total production (Barath *et al.,* 1990). The name maize is derived from the South American Indian Arawak-Carib word "Mahiz". It is also known as Indian corn or corn in America (Barath *et al.,* 1990). It was introduced into Nigeria in the 16th Century by the Portuguese (Bilgrami and Choudhery, 1998). The global production of maize is estimated to about 300million tonnes per year. 145million (or about 50 percent) of which are produced in USA alone (Osungbaro, 2009). In Nigeria its production is quite common in all parts of the country, from the North to the South with an annual production of about 5.6million tones (Piperno, 2011). The country's maize crop covers about 1million hectares out of nine million hectares it occupies in Africa (Harrigan and MeCance, 1998).

Maize is prepared and consumed in a multitude of ways which varies from region to region is from one ethnic group to another ethnic group. For instance, maize grains are prepared by boiling or roasting as paste ('eko'), abado', and 'elekute' in Nigeria and 'kenke' in Ghana, or as popcorn which is eaten all over West Africa. Traditional methods of preparations and uses of maize are restricted to definite localities or ethnic groups. This trend was also noted in the traditional preparation and uses of cassava (*Manihot esculenta crantz, Euphorbiaceae*) by Hunt *et al.,* (2004).

2.2. History of Maize

Pre-Columbian development Guilá Naquitz Cave in Oaxaca, Mexico is the site of early domestication of several food crops, including teosinte (an ancestor of maize) (Piperno,

2011). Most historians believe maize was domesticated in the Tehuacán Valley of Mexico. Recent research in the early 21st century has modified this view somewhat; scholars now indicate the adjacent Balsas River Valley of south-central Mexico as the center of domestication (Piperno, 2011). An influential 2002 study by Matsuoka *et al.* has demonstrated that, rather than the multiple independent domestications model, all maize arose from a single domestication in southern Mexico about 9,000 years ago. The study also demonstrated that the oldest surviving maize types are those of the Mexican highlands. Later, maize spread from this region over the Americas along two major paths. This is consistent with a model based on the archaeological record suggesting that maize diversified in the highlands of Mexico before spreading to the lowlands (Matsuoka *et al.,* 2002). Since then, even earlier dates have been published (Pagán-Jiménez *et al.,* 2015).

According to a genetic study by Embrapa, corn cultivation was introduced in South America from Mexico, in two great waves: the first, more than 6000 years ago, spread through the Andes. Evidence of cultivation in Peru has been found dating to about 6700 years ago. The second wave, about 2000 years ago, through the lowlands of South America.

The earliest maize plants grew only small, 25 millimetres (1 in) long corn cobs, and only one per plant. In Jackson Spielvogel's view, many centuries of artificial selection (rather than the current view that maize was exploited by interplanting with teosinte) by the indigenous people of the Americas resulted in the development of maize plants capable of growing several cobs per plant, which were usually several centimetres/inches long each (Spielvogel and Jackson, 2005). The Olmec and Maya cultivated maize in numerous varieties throughout Mesoamerica; they cooked, ground and processed it through nixtamalization. It was believed that beginning about 2500 BC, the crop spread through much of the Americas (Roney and John, 2009). Research of the 21st century has established even earlier dates. The region developed a trade network based on surplus and varieties of maize crops. Mapuches of south-

central Chile cultivated maize along with quinoa and potatoes in Pre-Hispanic times, however potato was the staple food of most Mapuches, "specially in the southern and coastal [Mapuche] territories where maize did not reach maturity (Bengoa and José, 2003). Before the expansion of the Inca Empire maize was traded and transported as far south as 40°19' S in Melinquina, Lácar Department. In that location maize remains were found inside pottery dated to 730 ±80 BP and 920 ±60 BP. Probably this maize was brought across the Andes from Chile. (Perez *et al.,* 2011). The presence of maize in Guaitecas Archipelago (43°55' S), the southernmost outpost of Pre-Hispanic agriculture, is reported by early Spanish explorers, however the Spanish may have misidentified the plant.

2.2.1. Columbian exchange

After the arrival of Europeans in 1492, Spanish settlers consumed maize and explorers and traders carried it back to Europe and introduced it to other countries. Spanish settlers preferred wheat bread to maize, cassava, or potatoes. Maize flour could not be substituted for wheat for communion bread, since in Christian belief only wheat could undergo transubstantiation and be transformed into the body of Christ (Torrejón *et al.,* 2013). Some Spaniards worried that by eating indigenous foods, which they did not consider nutritious, they would weaken and risk turning into Indians. "In the view of Europeans, it was the food they ate, even more than the environment in which they lived, that gave Amerindians and Spaniards both their distinctive physical characteristics and their characteristic personalities (Torrejón *et al.,* 2013). Despite these worries, Spaniards did consume maize. Archeological evidence from Florida sites indicate they cultivated it as well (Rhodes and Eagles, 1984).

Maize spread to the rest of the world because of its ability to grow in diverse climates. It was cultivated in Spain just a few decades after Columbus's voyages and then spread to Italy, West Africa and elsewhere (Rhodes and Eagles, 1984).

2.2.2. Naming

The word maize derives from the Spanish form of the indigenous Taíno word for the plant, mahiz. It is known by other names around the world. The word "corn" outside North America, Australia, and New Zealand refers to any cereal crop, its meaning understood to vary geographically to refer to the local staple In the United States, Canada, Australia, and New Zealand (Rhodes and Eagles, 1984). corn primarily means maize; this usage started as a shortening of "Indian corn (Boberg and Charles, 1984). Indian corn" primarily means maize (the staple grain of indigenous Americans), but can refer more specifically to multicolored " flint corn " used for decoration (Merriam-Webster, 2012).

In places outside North America, Australia, and New Zealand, corn often refers to maize in culinary contexts. The narrower meaning is usually indicated by some additional word, as in sweet corn, sweetcorn, corn on the cob, baby corn , the puffed confection known as popcorn and the breakfast cereal known as corn flakes .

In Southern Africa, maize is commonly called *mielie* (Afrikaans) or *mealie* (English), words derived from the Portuguese word for maize, *milho*. Maize is preferred in formal, scientific, and international usage because it refers specifically to this one grain, unlike corn, which has a complex variety of meanings that vary by context and geographic region. Maize is used by agricultural bodies and research institutes such as the Food Agency organisation (FOA). National agricultural and industry associations often include the word maize in their name even in English-speaking countries where the local, informal word is something other than maize; for example, the Maize Association of Australia, the Indian Maize Development

Association, the Kenya Maize Consortium and Maize Breeders Network, the National Maize Association of Nigeria, the Zimbabwe Seed Maize Association. However, in commodities trading, corn consistently refers to maize and not other grains.

2.3. Structure and physiology

The maize plant is often 3 m (10 ft) in height, though some natural strains can grow 13 m (43 ft) (Karl, 2012). The stem is commonly composed of 20 internodes of 18 cm (7.1 in) length. (Wellhausen and Edwin 1952). The leaf, which grows from each node, is generally 9 cm (4 in) in width and 120 cm (4 ft) in length. Ears develop above a few of the leaves in the midsection of the plant, between the stem and leaf sheath, elongating by around 3 millimetres (0.12 in) per day, to a length of 18 cm (7 in) with 60 cm (24 in) being the maximum alleged in the subspecies (Karl, 2007). They are female inflorescences, tightly enveloped by several layers of ear leaves commonly called husks. Certain varieties of maize have been bred to produce many additional developed ears. These are the source of the " baby corn " used as a vegetable in Asian cuisine (Rhodes and Eagles, 1984).

The apex of the stem ends in the tassel, an inflorescence of male flowers. When the tassel is mature and conditions are suitably warm and dry, anthers on the tassel dehisce and release pollen. Maize pollen is anemophilous (dispersed by wind), and because of its large settling velocity, most pollen falls within a few meters of the tassel. Elongated stigmas , called silks , emerge from the whorl of husk leaves at the end of the ear. They are often pale yellow and 18 cm (7 in) in length, like tufts of hair in appearance. At the end of each is a carpel, which may develop into a "kernel" if fertilized by a pollen grain. The pericarp of the fruit is fused with the seed coat referred to as " caryopsis ", typical of the grasses, and the entire kernel is often referred to as the " seed". The cob is close to a multiple fruit in structure, except that the individual fruits (the kernels) never fuse into a single mass. The grains are

about the size of peas, and adhere in regular rows around a white, pithy substance, which forms the ear. The maximum size of kernels is reputedly 2.5 cm (1 in) (Grobman and Alexander 1961). An ear commonly holds 600 kernels. They are of various colors: blackish, bluish-gray , purple , green, red, white and yellow. When ground into flour , maize yields more flour with much less bran than wheat does. It lacks the protein gluten of wheat and, therefore, makes baked goods with poor rising capability. A genetic variant that accumulates more sugar and less starch in the ear is consumed as a vegetable and is called sweet corn. Young ears can be consumed raw, with the cob and silk, but as the plant matures (usually during the summer months), the cob becomes tougher and the silk dries to inedibility. By the end of the growing season, the kernels dry out and become difficult to chew without cooking them tender first in boiling water (Torrejon *et al.,* 2013).

2.4. USES OF MAIZE

2.4.1 Human Food

Maize and corn meal (ground dried maize) constitute a staple food in many regions of the world. Maize is central to Mexican food. Virtually every dish in Mexican cuisine uses maize. One form of grain or cornmeal, maize is the main ingredient of tortillas, tamales, pozole, atole and all the dishes based on them, like tacos, quesadillas, chilaquiles, enchiladas, tostadas and many more. In Mexico even a fungus of maize known as *Huitlacoche* is considered a delicacy. Maize which is a major source of starch (Torrejon *et al.,*2013), is a major ingredient in home cooking and in many industrialized food products. Maize is also a major source of cooking oil (corn oil), and of maize gluten. Maize starch can also be hydrolyzed and enzymatically treated to produce syrups, particularly high fructose corn syrup, and sweetener; and also fermented and distilled to produce grain alcohol. Sometimes maize is used as the starch source of beer.

2.4.2 Chemicals

Starch from maize can also be made into plastics, fabrics, adhesives, and many other chemical products. The corn steep liquor, a plentiful watery by product of maize wet milling process, is widely used in the biochemical industry and research as a culture medium to grow many kinds of microorganisms (González *et al.,* 1999).

2.4.3 Medicinal Uses

A crop which is highly edible and nutritious as maize, also has some medicinal uses among the local people. It is used to cure many diseases, which it had over the years proved to be very effective.

These include:

1. Water filtered through charcoal obtained from maize stalk can be used as a treatment to cure gonorrhea (Abdulrahman, 1997).
2. An infusion obtained from stigma of maize inflorescence can be used for treatment of diseases of the urinary tract or passage (Abdulrahman 1997).

Maize is consumed in many forms in different parts of the world, from maize grits, polenta and corn bread to popcorn and products such as maize flakes (Rooney and Serna Saldivar, 1987). The grain is fermented to give ogi in Nigeria (Oke, 1967) and other countries in Africa (Hesseltine, 1979) and is decorticated, degermed and precooked to be made into arepas in Colombia and Venezuela (Rodriguez, 1972).

2.5. Grain microorganisms

In terms of microorganisms, fungi and their associated secondary metabolites known as mycotoxins are of high concern in grain shipments or storage facilities due to the production of mould, odours, the presence of microbial 'hot-spots', and the production of secondary metabolites which can lead to subsequent poisoning of food and animal feed, thus negatively impacting food safety (Tefera *et al*., 2011).

There are a number of postharvest fungi that can attack and cause damage to grain, and they can be divided into two groups: field fungi and storage fungi (Miller, 1995). Field fungi may modify the structure and quality of seeds or grains. These cause damage to the grain before harvest and can generally be detected by routine assessment. In general, field fungi do not occur in storage if the grain is stored at appropriate moisture contents and temperatures (Christensen and Kaufmann, 1965). Storage fungi are those that cause damage to grain during storage and usually do not occur at a serious level prior to harvest (Muir and White, 2000).

The mycoflora of stored grains predominantly consist of the ubiquitous mould genera *Aspergillus*, *Alternaria*, *Cladosporium*, *Fusarium*, *Mucor*, *Rhizopus* and *Penicillium* (Mathew *et al*., 2010). They are usually introduced into the stored grain as spores in minute quantities during handling and storage. Other microorganisms such as certain bacteria can also colonise grain. These bacteria mainly belong to the families *Pseudomonadaceae, Micrococcaceae*, *Lactobacillaceae* and B*acillaceae* (Laca *et al*., 2006). In Australia, Europe, and the US *Salmonella* spp., *Escherichia coli*, and *Bacillus cereus* are also present in wheat and flour at low levels (Ottogalli and Galli, 1979). The presence of these bacteria and fungi and their adverse effects can be compounded further by insect activity (Jayas *et al*., 1994) and if the moisture content rises above 13%-15 %, wheat moulds begin to develop (Jay, 1996). Protection can be achieved by decreasing grain temperature and controlling moisture

migration with aeration or by using ambient or refrigerated air (Collins and Conyers, 2010). Mycotoxins produced by some fungi cause a large number of diseases annually, from liver and esophageal cancer, acute toxicosis, immune suppression, and can also stunt growth in 13 children (Wu *et al*., 2011). The majority of infections of animals (e.g chronic aflatoxicoses) on farms are caused by mycotoxins being present in poor quality feed (Zain, 2011). For example, Aflatoxin B1 is highly toxic and is a potent carcinogen to both humans and animals. Fumonisin B1 (FB1) is produced by *Fusarium moniliforme* and can cause equine leucoencepha-lomalacia (Kellerman *et al*. 1990) and porcine pulmonary edema these infections were observed in animals after they had consumed contaminated corn. Very few cases of human disease caused by mycotoxins have been recorded in Australia but have been recorded in animals (Blaney, 2007). It is important to note that fungi can produce extremely high mycotoxin concentrations in small pockets of grain, which have the potential to contaminate larger amounts of grain at levels exceeding acceptable limits for domestic and export markets (Magan *et al*., 2003).

2.5.1. Detection and identification of fungi in grain

The majority of fungal detection methods in food sources require representative sampling and dissection of the food sample before fungal growth can be detected (Magan and Evans 2000; Paolesse *et al*., 2006). As the techniques used often provide only presence/absence data rather than a direct indication of the extent of fungal invasion, if contaminants are found, the food product may be discarded or downgraded (for animal feed) (Golob, 2007). Traditionally, methods for the identification of stored fungi were based on the morphology of cultures and microscopic features such as spores and fruiting structures. Methods for the detection of mould contamination and mould growth using agar media have a number of disadvantages. Counting of colony forming units is slow and not related to actual fungal activity. Representative sampling is also difficult, as this method requires a number of days from

isolation to identification and is time consuming and expensive. Furthermore, culture medium preparation, inoculation of plates, colony counting and biochemical characterization are labor intensive

2.6. PAP

Pap is fermented cereal porridge from West Africa, typically made from maize or millet. Traditionally, the grains are soaked in water for up to three days, before wet milling and sieving to remove husks. The filtered cereal is then allowed to ferment for up to three days until it sour. It is then boiled into pap, or cooked to make a stiff porridge. The fermentation of "pap" is performed by various lactic acid bacteria including *Lactobacillus* Spp. and various yeasts including *Saccharomyces* and *Candida* Spp.

2.6.1. Pap Manufacturing

Traditional process of making pap has a number of slight variations described by several authors. Pap is traditionally prepared in batches on a small scale of two or three times a week, depending on demand. The clean grain is steeped in water for one to four days to soften. Once soft, it is grounded with a grinding stone, pounded in a mortar or grounded with a power mill. The bran is sieved and washed away from the endosperm with plenty of water. Part of the germ is also separated in this operation. The filtrate is allowed to ferment for 24 to 72 hours to produce slurry which when boiled gives the ogi porridge. Pap is usually marketed as a wet cake wrapped in leaves, or it may be diluted to 8 to 12 percent solids in water and boiled into a pap or cooked to a stiff gel (Akingbala, 1981)

Akinrele (1970) reported that the souring of the maize took place spontaneously without the addition of inoculants or enzymes. He identified the organism involved in this unaided fermentation and investigated their effects on the nutritive value of the food. The moulds he identified are *Epholosporium, Fusarium, Aspergillus* and *Penicillium* species and the aerobic

bacteria as *Corynebacterium* and *Aerobactor species*, while the main lactic acid bacterium he found was *Lactobacillus plantarum*. There were also yeast*: Candida mycoderma, Saccharomyce cerevisiae* and *Rhodotorula sp.* Although "ogi" is supposed to have an improved vitamin-B contents the result observed are quite variable, at least for thiamine, riboflavin and niacin. Banigo and Muller. (1972) identified the carboxylic acids of pap fermentation. They found 11 acids, with lactic, acetic and butyric acids being the most important. The ogi making process is quite complex, and the porridge can also be prepared from sorghum, rice, millet and maize. Therefore, laboratory procedures have been developed to learn more about the process and introduce changes to convert the grains to food more efficiently. These have been described by Akingbala *et al.,* **(**1990). Whose studies have been useful in evaluating varieties of cereal grains for their efficiency in making "ogi" from whole male are kernels (79.1 %) and dryed milled flour (79.8 %)

The commercial manufacture of 'ogi' does not differ substantially from the traditional method. Modifications have been introduced or added, such as the dry milling of maize into a fine meal or flour and subsequent inoculation of the flour-water mixture with a culture of *lactobacilli* and yeast. In view of the importance of 'ogi' in the Nigerian diet, large scale production is indicated. The material could be dried and packaged in polythene bags for a good shell life. This is why there is some problem in achieving a controlled fermentation with pure cultures. Some of the modification which include spray drying the slurry or drum drying.

2.6.2. Nutritional and Chemical Changes of Pap And Other Fermented Maize Products

2.6.2.1. Chemical changes

The process of fermenting maize, sorghum, or millet to produce pap not only removes parts of the maize kernels such as seed-coat and the germ, but also involves washing, sieving and decanting all of which induce changes in the chemical composition and nutritive value of the final product. Akinrele(1970) reported on specific nutrients of a number of 'pap' samples produced in different ways unfermented and fermented with *Aerobactor cloacae, Lactobacillus plantarum* and a mixture of the two bacteria. He also compared the values found with those from the traditionally fermented products. Judging from the ratio of amino nitrogen to total nitrogen, the author reported that protein was degraded to a very small amount by any bacterial species. When compared with the unfermented 'pap' *Aerobacter cloacae* appeared to synthesis more riboflavin and niacin ,which did not take place with *L. plantarum*. Traditionally produce 'ogi' had more thiamine and slightly lower the values of riboflavin and niacin than that made with maize and *A. cloacae.*

Akinrele (1970) and Banigo and Muller(1972) reported the carboxylic acids in ogi and found lactic acid in greatest concentration (0.55 percent) followed by acetic acid (0.99percent) and smaller amounts of butyric acid. The latter investigators suggested levels of 0.65 percent for the lactic acid and 0.11 percent for acetic acid, which are responsible for sour taste as goals for flavor evaluation (Dellicour and Lecocq, 2013). reported on the proximate composition of Ogi made from common whole maize which were uncooked, and freeze-dried or cooked and freeze-dried after fermentation. Changes were relatively small in all major nutrients, with a slight increase in fiber and a decrease in ash content when compared with whole maize. These authors also reported on amino acid content, they found no differences maize flours and 'ogi' for all amino acids including the essential ones. The pap samples, however had about twice the amount of serine and somewhat higher values for glutamic acid. (Girotti *et al.,* 2012). reported that ogi processing did not decrease the protein

content of maize, but total and available lysine were significantly reduced. On the other hand, tryptophan levels were more stable and in two samples increased, probably because of fermentation. These authors also found an increase in neutral detergent fibre and ash but no change in ligin. Akingbala *et al.* (1990) found a decrease in protein, ether extract, ash and crude fiber in ogi as compared with maize that was processed as a whole grain or dry milled.

2.6.2.2. Nutritional Changes

The nutritional evaluations of Ogi and other maize fermented products are not readily available. Adeniji and Potter (1978) found a substantial decrease in protein quality of drum dried common maize ogi, which they ascribed to the drying process. These same authors reported significant losses on lysine. Several authors have more recently tested maize and sorghum and reported that fermentation improved the nutritional value of the product. Akinrele and Bassir (1967) found protein utilization to those values in whole maize, even though some increase in thiamine and niacin was obtained. It has been indicated that some of the microorganisms responsible for 'ogi; fermentation, such as *Enterobacter cloacae* and *Lactobacillus plantarum* use some of the amino acids for growth. This together with the elimination of the germ from Kernels explains that the very low protein quality of ogi and similarly produced maize products.

2.6.2.3. Microbial Properties of Pap

Three distinctive fermentative phases are characterized with pap production. At steepings Gram negative organisms predominates especially *Achromobacter* and *Klebsiella spp*. Following the milling and sieving, Gram negative organisms and lactic acid bacteria especially *Streptoccus* spp dominates. The final stage of souring is dominated by non-homofermentative lactic bacteria especially *Lactobacillus plantarum* and *Pediococcus gunther* leads to the involvement of yeast as a minority component. *Saccharomyces*

cerevisiae dominates the steeping stage while *Candida* survives in the finished product. Fermentation temperature is the major factor affecting the type of organisms involved in the production process. The lactic acid bacteria *Lactobacillio sp, Corynebacterium sp* and *Enterobacter sp* were among the major organisms responsible for the fermentation and nutritional improvement of pap (Akinrele 1970).

At 15^0C, Gram negative organisms survive in the products at the end of seven days; whereas at 33^0C and 37^0C, the flora is more heterogeneous and the end product is accompanied by an odd odour and taste. Large fermentation rods suggestive of *Bacillus spp*. are also targeted to be the causal agent of proteolysis and the putrial colour of 'ogi'. Also, the gas evolution that is found during steeping is attributed to the presence of *Klebsiella aerogenes*.

2.6.2.4. Biochemistry of Pap

When the grain is fermented, there is an increase in pH. The raw 'ogi' contains much less protein than the parent cereal because some soluble proteins are lost in steeping, washing with water and during mashing. Acid reacting substances are present during mashing. Acid reacting substances are also present during ogi fermentation and increases as the fermentation progresses (Crous *et al.,* 2015).

Lactic acid is the primary volatile acid of ogi fermentation, acetic acid is the main volatile acid followed by butyric acids other volatile acids of fermentation are formic acid, propionic acid, isobutyric acid, isohexonic acid etc. during steeping of water it contains a higher amount of volatile acids unlike finished products, because the bulk of the acid produced in the later stages of the fermentation is leached out into the water. These acids appear in form of filing on the surface of the water (Darby and Caddick, 2007).

2.7 Microbial Contamination at Different Stages of Ogi Production

The outbreak of infectious and communicable diseases in tropical parts of the world is primarily as a result of food poisoning due to microbial contamination (Jay, 2005). They are often responsible for acute gastroenteritis, abdominal discomfort and pain and diarrhea in infants and young adults.

Maize grains were almost surfaced sterile prior to soaking. The isolated *Staphylococcus aureus* in few maize samples could have arisen from contaminated sacks used for storage and transportation of produce. Onovo and Ogaraku (2007) discovered some bacteria and fungi on exposed tigernet (*Cyperus esculentus* L.) before processing. The presence of *Aspergillus flavus, A. niger, Penicillium oxalicum, Fusarium oxysporium, Rhizopus stolonifer, Saccharomyces cerevisiae Candida albicans, Escherichia coli, Kebsiella aerogenes and Staphylococcus aureus* in water from the reservoirs suggests an extremely poor storage system deplorable sanitary conditions availab le at the three sites and multiple source of contamination due to open access to the reservoirs. Exposure of water to direct rays from the sun provided the required warmth and physical condition for growth and physical condition for growth of these organisms. Ozoh and Kuyanbana (1995) and Osho and Fagade (2000) equally verify water as the source of *Shigella spp* and *E. coli* in maize and other cereals porridge. Contaminated water was linked as the main source of *Vibrio cholera* infection in some population in Iran. Oranusi *et al.* (2007) estimated 2-3 log10 coliforms per $100gml^{-1}$ of cooked maize porridge and linked contamination to the water used during the washing and soaking of maize grains. Heavy presence of *E. coli, Klesbsiella pneumonia* and *Streptococcus spp.* was reported in some food around the University of Ghana campus. The introduction of *Salmonella sp., Rhizopus sp.,* and *Staphylococcus aureus* in some foods products have been linked to the presence of phytoxocin.

The infinite and open access to the water tanks allowed cross contamination of the cooking utensils and bowls subsequent re-contamination of products at the later stages of

production. The body swabs and underneath of nails contained substantial counts of *Staphylococcus aureus* and *Lactobacillus plantarum*, *E. coli, Klebsieela aerogenes and Saccharomyces cerevisiae*. The muslin clothes used in sieving the shaft were stained and soiled and often reused without thorough washing. The wrapping leaves and polythene bags were not sufficiently rinsed or sterilized before use. Omemu and Adeosun, (2010) observed similar unhygienic practices among attendants and vendors at some production sites in Abeokuta, Nigeria. Air was laden with *Aspergillus flavus, Penicillium oxalicum* and *Rhizopus stolonifer* and served as a source of re-contamination of the finished products. Wacher *et al.* (1993) linked the contamination of freshly prepared pozol, traditional Mexican fermented maize dough to the surrounding air. The growth of bacteria (*Escherischia coli* and *Klebsiella aerogomes)* declined significantly in fully fermented wet paste as rightly observed by Byaruhanga *et al*. (1999) for *Bacillus cereus* after 24 hours fermentation. Also Mensah *et al.* (1990, 1991) observed a significant inhibition in the growth of some gram negative bacteria. Chukeatirote *et al.* (2010) observed an exponential increase in the population of bacteria and fungi with increased pH and fermentation time of grain.

However, a re-contamination at latter stages of production by these enteric bacteria as observed could be linked to water as it was used repeatedly during preparation. Odugbemi *et al. (*1993) reported an increase in the level of faecal coliforms in cooked ogi under 9hours storage conditions and suggested a probable re-introduction during storage. A similar conclusion was held by Sanni *et al.,* (2002) for the rise in the population of yeast from 1.0 $cfug^{-1}$ to 5.36 $cfug^{-1}$ after 12 hours of fermentation. Alalade and Adeneye (2007) observed a significant correlation between pH and coliform bacterial count in wara cheese during fermentation process. Poor handling by vendors or sellers was rightly suggested by Wacher *et al.,* (1993) for the significant increase in enteric bacteria in freshly prepared pozol. On the other hand, the growth of *Lactobacillus plantarum* was unhindered at the different stages of

production even after 48 hours of fermentation. Relatedly, an exponential increase in growth of some lactic acid bacteria was earlier reported by Kunene *et al.,* (1999) in both fermented and cooked maize porridge. During the preparation of pap, the critical points of contamination includes; the point of soaking the grains, milling and wrapping the products. Effective and good manufacturing practices (GMP) as recommended by Amoa-Awua *et al.,* (2007) would help eliminate contaminants for improved table quality and assure the health of consumers.

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS USED

Maize sample, Weighing balance, Pipette, Conical Flask, Beaker ,Petri dish, Test tube, Foil paper, Autoclave, Incubator, Staining rack, Bijou bottles.

3.2 STUDY AREA

The samples used in this study were collected within Benin metropolis. Six maize and pap samples were collected from New-Benin market area Benin city Edo state Nigeria.

3.3 Sample collection

The samples were collected with the usual measuring cup used in the various markets by the vendors. The samples from each vendor were placed in sterile clear seal bags provided for sampling. The packaged and labelled samples were immediately transported in a clean air tight food flask to the laboratory for mycological analyses.

3.4 Sterilization of materials

The glass wares used for this study were thoroughly washed with detergents and rinsed with distilled water. The glass wares such as conical flask, beakers, test tubes and pipettes were wrappezd with aluminum foil and appropriately sterilized in the hot air oven at 160ºC for one hour (Colomb *et al.,* 2008).

3.5 Culture media

The medium used was Potato Dextrose Agar (PDA). The medium was prepared according to manufacturer's specifications. Appropriate grams of the powder were dissolved into 1000 ml of distilled water. The mixture was thoroughly shaken and allowed to boil. It was sterilized by autoclaving at 121°C for 15 minutes. After the sterilization, it was allowed to cool at 45°C before dispensing into sterile Petri dishes.

3.6 Isolation and identification of pure isolates, enumeration of microorganisms

3.6.1 Isolation of Fungi

40gm of maize obtained from New Benin market were washed in 60mls of sterile distilled water (H_20) for 10mins by hand shaking in a 200ml conical flask separately respectively. The wash of the maize were used as inoculum to be used in a pour plate technique for the isolation of fungi. For pap analysis 1 ml of the pap samples were dissolved in 9ml and a tenfold serial dilutions of the samples from 1:10 (10^{-1}) to 1:100000 (10^{-5}) were carried out. Aliquots of 0.1ml from the 1:100 (10^{-2}) dilution for each samples were plated (Begerow *et al.,* 2010)

Potato Dextrose agar (PDA) amended with antibacterial agent was used, in each case the wash of the maize served as the inoculum. 2-3 drops of the inoculum was poured into the Petri dish and a check cooled PDA poured and swung to mix. triplicate plates were incubated at room temperature 28 ± 2^0C on the laboratory bench for 3-5 days under photo period of light. The culture plates did not grow well after 5 days of incubation at room temperature (28 ± 2^oC). The plates were further incubated for 7 days to observe for proper growth concerning color changes and sporulation of cultures while awaiting the slow growers. Fungi isolated were identified using the scheme of Barnett and Hinter, 2005.

3.6.2 Identification and Characterization of Isolates

The identification of fungi isolates was based on their morphological and cultural characteristics of Barnet and Hunter, 1974. Agar cultures of each isolate were used in determining their cultural characteristics. 3 to 5 days old cultures of fungi plates were used to study the culture, plate culture reversed and nature of growth. The features examined in the colonies include: Nature of growth, plate culture reverse, shape of spores and attachment, colour of spores and culture.

3.6.3 Colony forming units

The number of viable micro-organisms in the sample was calculated from the number of colonies formed and the volume of inoculums and the dilution factor expressed in colony forming unit. Aydin *et al.,* 2009)

3.6.4 Frequency of Occurrence.

The fungi isolated from samples were placed according to the type of samples from which they were isolated. This was done to qualitatively analyze the presence of the isolates and the quality of the samples in terms of presence or absence of fungi (Beckett, 2011).

CHAPTER FOUR

RESULT

Table 1a and 1b shows the cultural and morphological characteristics of fungal isolates from maize and pap samples. Isolated fungi are *Penicillium oxalium, Penicillium italicum, Aspergillus niger, Aspergillus flavus, Mucor mucedo, Penicillium oxalium, Cladiosporium* sp., *Saccharomyces* spp., *Mucor* sp., *Clavispora* spp., *Cryptomonas* spp. *Saccharomycodes,* spp. and *Galcatomyces* spp.

Table 2 shows the mean heterotrophic fungi colonies count for maize and pap. The total fungi count of maize samples collected from different markets ranged from $1.4 \times 10^6 \pm 0.9$ – $5.7 \times 10^5 \pm 0.7$ cfu/g, while fungal count of pap samples ranged from $1.40 \times 10^6 \pm 0.7$ – $5.1. \times 10^5 \pm 0.7$ cfu/g.

Table 3. shows the percentage frequency of occurrence and the distribution pattern of fungi isolates obtained from differe1nt samples of maize and pap from different locations. A total of 13 fungal isolates were identified.

Table 1: Cultural and microscopic characterization of fungal isolates from pap and maize

Cultural Characteristics													
1	**Growth form**	Greenish, woolly with profuse growth	Black, woolLy with profuse growth	Green, non-luxuriant with concentric ring	Whitish, mucoid edges, entire raised	Army green and entire, non-luxuriant with concentric ring	Green with white marring, non-luxuriant with concentric ring	Yellow muccoid, edges entire raised	white muccoid, edges entire raised	White extensive woolly cottony with coenocytic hyphae	Grayish black/ Dark olive green, wooly profuse growth	Creamy splashes muccoid, edges entire raised	Creamy muccoid, edges entire raised
2	**Colour of reverse plate**	Creamy	Dark	Orange	Creamy	Orange	Orange	yellow	white	Whitish	Grayish	Creamy	Creamy
Microscopy													
3	**Hyphae**	Septate	Septate	Septate	No hyphae	Septate	Septate	Non-hyphae	Non-hyphae	Non-septate (young) Septate (old)	Septate	Non-hyphae	Non-hyphae
4	**Conidiophores**	Non-septate terminating in clavate swelling	Non-septate terminating in globose swelling	Septate arise from a mycelium singly, branched near apex	Non conidiophores	Septate arise from a mycelium singly, branched near apex	Septate arise from a mycelium singly, branched near apex	Non-conidiophore	Non-conidiophore	Non-septate, long erect usually unbranch single from coenocytic hyphae	Tall, upright branches near apex bearing conidia	Non-conidiophore	Non-conidiophore
5	**Conidia**	Present, globose in dry basipetal chains	Present, one-celled, globose in dry basipetal chains	Present one-celled hyaline, globose brightly coloured basipetal	Ellipsoid cells with ends on the sides	Present one-celled hyaline, globose brightly coloured basipetal	Present one-celled hyaline, globose brightly coloured basipetal	Ellipsoid cells	Ellipsoid cells	Present, hyaline one-celled, globose non-motile	Present, cluster or single (1 or 2 celled)	Ellipsoid cells	Ellipsoid cells
6	**Stolen**	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent, presence of coenocytic hyphae	Absent	Absent	Absent
7	**Rhizoid**	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
8	**Spore colour**	Green	Dark	Colourless	Whitish	Colourless	Colourless	Pinkish	Pinkish	Whitish	Grayish black	Pinkish	Pinkish
9	**Spore attachment**	Radiate from the entire surface at the tip	Bear phialides at the apex with conidia at the tip	Phialids which pinch off conidia in dry chains at the tip	Buds growing on the side	Phialids which pinch off conidia in dry chains at the tip	Phialids which pinch off conidia in dry chains at the tip	Single cells	Single cells	Tip of sporangiophore in the sporangia	Terminal, bear phialides at the apex terminating in conidia	Single cells	Single cells
10	**Tentative Identity**	*Aspergillus flavus*	*Aspergillus niger*	*Penicillium* sp.	*Saccharomyces* sp.	*Penicillium oxalicum*	*Penicillium italicum*	*Saccharomycodes sp.*	*Galactomyces sp.*	*Mucor* sp. (Mucedo)	*Cladosporium* sp.	*Cryptomonas* sp.	*Clavispora sp.*

Table 2 : Total fungal count of maize and pap samples

Samples	MEAN±S.E (cfu/g)
A	**$4.0 \times 10^5 \pm 0.7$**
B	**$1.4 \times 10^6 \pm 0.9$**
C	**$5.7 \times 10^5 \pm 0.7$**
D	**$1.4 \times 10^6 \pm 0.7$**
E	**$5.1 \times 10^6 \pm 0.8$**
F	**$2.0 \times 10^6 \pm 0.8$**

Values are triplicate determination

Key ;

A = guinea corn

B= yellow corn

C= white corn

D= Guinea corn pap,

E= Yellow pap,

F= white pap

Table 3: Percentage frequency of occurrence of fungi isolates obtained from different samples of maize and pap

Isolates	Frequency	Percentage frequency	Sample locations					
			A	**B**	**C**	**D**	**E**	F
Aspergillus infer	2	5.4	+	-	+	-	-	-
Aspergillus flavus	3	8.1	+	+	+	-	-	-
Pencillium oxalium	1	2.7	+	-	-	-	-	-
Pencillium italium	1	2.7	-	-	+	-	-	-
***Pencillium* sp.**	3	8.1	+	-	-	-	+	+
Mucor sp.	4	10.8	+	+	+	-	+	-
Mucor mucedo	1	2.7	-	-	+	-	-	-
Cladosporium sp.	3	8.1	+	+	+	-	-	-
***Saccharomyces* sp.**	3	8.1	+	+	+	+	-	-
***Cyrptomonas* spp.**	3	8.1	-	-	-	+	+	+
***Clavispora* sp.**	6	16.2	+	+	+	+	+	+
***Galactomyces* sp.**	4	10.8	-	+	+	-	+	+
Saccharomycodes	2	5.4	-	-	-	+	+	-
Fungi frequency	37		8	6	9	4	6	4
Fungi % frequency		99.9	21.6	16.2	24.3	10.8	16.2	10.8
Fungi diversity	5		4	4	5	1	3	2
Fungi % diversity		13.5	80	80	100	20	60	40

Key; A = guinea corn, B= yellow corn, C= white maize, D= guinea corn pap, E= yellow pap, F= white pap.

Key; - = absent, + = present.

CHAPTER FIVE

DISCUSSION

The main of this study was to isolate and identify fungi from maize and its products (pap) Microorganisms play both essential and deleterious roles in food products. In the fermentation industry, the attributes of the food products produced is largely due to the type, age, composition of the microorganisms employed. To a large extent, both population and diversity play a role in the fermentation of products.

The Total heterotrophic fungi count (TFBC) was used as important indicator of the microbial quality of maize and pap samples. A total of 6 samples from six vendors were cultured for total heterotrophic fungi counts on different bacteriological media. The total fungi count of maize and pap samples ranges from $4.0 \times 10^5 – 1.4 \times 10^6$ cfu/g and 1.40×10^6 - 2.00×10^6 cfu/g cfu/g respectively. The CFU/ml values obtained shows that there is a significant differences between total heterotrophic counts of samples collected from different locations. The mean THFC obtained was lower than that reported by Osho, *et al.* (2010) (1.94×10^7 cfu/g to 2.44×10^7 cfu/g) and Bello, *et al.* (2016) (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 3.2×10^4 cfu/ml to 4.7×10^6 cfu/ml (Badmos *et al.,* 2014).

The morphological, cultural and biological characteristics of microbial isolates revealed the following fungi genera genera; *Penicillium oxalium, Penicillium italicum, Aspergillus niger, Aspergillus flavus, Mucor mucedo, Penicillium oxalium, Cladiosporium* sp., *Saccharomyces* spp., *Mucor* sp., *Clavispora* spp. and *Cyptomonas* spp., *Saccharomycodes, Galcatomyces*. This results is not with study done by Larone, (1998) isolated *Fusarium spp., Penicillium* spp., *Rhizopus* spp., *Aspergillus* spp. and *Mucor* spp. from maize and pap samples. A total of 37 fungal isolates were identified. The percentage

occurrence of the isolates from maize and pap in this study showed that *Clavispora* spp. (16.2 %), *Mucor* ***sp*** (10.8 %). *Galactomyces* sp. (10.8) as the most predominant fungal isolate. Distribution of fungi isolates in collected samples of maize and pap showed that the maize samples had higher fungi count than the pap samples. Sample A (21.6 %), C (24.3 %) as the most contaminated isolates, while sample D (10.8) and F (10.8) was the least contaminated.. The result of fungi biodiversity showed that maize samples A (4), B (4) and C (5) showing more fungi diversity than pap samples D(1), E(3), F(2).

The low fungi isolates in pap samples might have been as a result of fermentation processes

Larone, (1998). Recorded the percentage prevalence of in the analyzed maize samples as: *Fusarium spp* (36.4%), followed by *Penicillium* spp. (33.4%),n *Rhizopus* spp. (15.2%), *Aspergillus* spp. (9.09%) and *Mucor* spp. (6.06%), Kohajdová *et al.* (2007) found the prevalence *Saccharomyces* spp., *Mucor* sp., *Clavispora* spp., *Saccharomycodes, Galcatomyce.*

5.2 CONCLUSION

This study revealed the common fungi flora of maize and pap samples sold in local markets in Benin City. The diversity of fungal genera associated with maize and pap may be dependent upon environmental conditions at the time of harvest, processing and the difference between the storage structures. It is therefore recommended that further studies be undertaken to understand the role of each fungi generaa isolated in this study and their source of contamination.

REFERENCES

Adebolu, S. A, Banigo, E. O. and Torre, S. I. (2007). Characteristics and importance of fufu and ogi. *Journal of food science and Nutrition* **8** (5) 449-553.

Adhikari, E . C., Teniola, O. D. and OLukoya, O. K. (1994). Properties of ogi powder made from normal fortified and opaque corn. *Journal of Food Science* **43** (21): 154-162.

Akingbala F. , Badmos, A. H. A., Kayode K. (1990). Occurrence of *fusarium* species and their pycotoxins in maize silage *Global Journal of Bio-Science and Biotechnology* **3**(2): 128- 132.

Banigo, E. O., Muller, H. G. and Odunfa, S. A. (2002). Comparative evaluation of corn. *Journal of Food Science Technology* **45**(50): 217-221.

Barath, H., Knabe, O. and Lepom, P. (1990). Occurrence of *fusarium* species and their pycotoxins in maize silage: studies on the *fusarium* infestation of maize silage plants. *Arch Animal Nutrition* **40**(1): 397- 298.

Beckett, S. (2011). Insect and mite control by manipulating temperature and moisture before and during chemical-free storage. *Journal of Stored Products Research* **47**(4): 284-292.

Bengoa B.B and José R. D. (2003). Historia de los antiguos mapuches del sur (in Spanish). Santiago: Catalonia. pp. 199–200.

Bilgrami, K. S. and Choudhery, A. K. (1998). *Mycotoxins and food Safety*, Marcel Dekker Inc., New York pp 399.

Blaney, B. J. (2007). Mycotoxins in Australian maize: a risk assessment. *Department of Primary Industries and Fisheries, Locked Mail Bag* (4) 1-19.

Boberg H. and Charles F. (2010). The English Language in Canada: Status, History and Comparative Analysis. Cambridge University Press p109.

Christensen, C. M., and Kaufmann, H. (1965). Deterioration of stored grains by fungi. *Annual Review of Phytopathology* **3**(1), 69-84.

Collins, D., and Conyers, S. (2010). The effect of sub-zero temperatures on different life stages of *Lasioderma serricorne* (F.) and *Ephestia elutella* (Hübner). *Journal of Stored Products Research* **46**(4): 234-241.

Common Corn Questions and Answers Archived May 1, 2012, at the Wayback Machine, Iowa State University of Science and Technology, Agronomy Extension, 2011

Crous, P. W., Müller, M. M., Sánchez, R. M., Giordano, L., Bianchinotti, M. V., Anderson, F. E. and Groenewald, J. Z. (2015). Resolving *Tiarosporella* spp. allied to Botryosphaeriaceae and Phacidiaceae. *Phytotaxa,* **202**(2): 73-93.

Darby, J.A., and Caddick, L.P. (2007). Review of Grain Harvest Bag Technology under Australian Conditions. Commonwealth Scientific and Industrial Research Organisation (CSIRO) Entomology, Melbourne, Victoria, Australia. Technical Report – No. 105

Dellicour, S. and Lecocq, T. (2013). GCALIGNER 1.0: An alignment program to compute a multiple sample comparison data matrix from large eco-chemical datasets obtained by GC. *Journal of Separation Science* **36**(19): 3206-3209.

Did man follow plants or did plants follow man? Tracks of prehistoric man and ways of contact in the Americas according to cultivated plants. Case study – Maize (translated from Portuguese)". Yumpu. 2015. Retrieved October 13, 2015.

Girotti, J., Malbran, I., Lori, G. and Juarez, M. (2012). Early detection of toxigenic *Fusarium graminearum* in wheat. *World Mycotoxin Journal* **5**(2): 143-152.

Golob, P. (2007). *On-farm mycotoxin control in food and feed grain* (Vol. 1). Food and Agriculture Organization

González, H., Martínez, E. J., Pacin, A. and Resnik, S. L. (1999). Relationship between *Fusarium graminearum* and *Alternaria alternata* contamination and deoxynivalenol occurrence on Argentinian durum wheat. *Mycopathologia* **144**(2): 97-102.

Grobman, Alexander (1961). Races of Maize in Peru .

Hunt, J., Boddy, J., Randergon, P. F. and Rogers, H. J. (2004). An evaluation of DNA approaches for the study of fungal diversity in grassland soils. *Journal of Microorganism and Ecology* **160**(1): 385-388.

Jaris, R. C. (2001). Medicinal importance of maize crops. *Journal of Medicine* **2**(5): 16-25.

Jay, J. M. (1998). *Food Spoilage in Modern Food Microbiology.* 4th Edition, Chapman and Hall Inc. New York, p. 195.

Jay, A. K. (2005). The effect of processing methods on the levels of lysine tryptophan and the general acceptability of ogi processed using starter culture. *Journal of Food Microbiology* **24** (31): 239-248.

Jay, M. M. (1996). *Modern Food Microbiology* (5th ed.). New York, USA: Chapman & Hall, International Thompson Publishing, pp. 661.

Jayas, D. S., White, N. D. and Muir, W. E. (1994). *Stored-Grain Ecosystems* (Vol. 39): CRC Press.ft

Karl, J. R. (2007). Jala Maize is Small. Maize Genetics MNL . **89** : 3-6.

Karl, J. R. (2012). The maximum leaf number of the maize subspecies. *The Maize Genetics Cooperation Newsletter* **86**: 4-5.

Kellerman, T. S., Marasas, W., Thiel, P., Gelderblom, W., Cawood, M. and Coetzer, J. (1990). Leukoencephalomalacia in two horses induced by oral dosing of fumonisin B1. *The Onderstepoort Journal of Veterinary Research* **57**(4):269-275.

Kohajdová, Z. and Karovičová, J. (2007). Fermentation of cereals for specific purpose. *Journal of Food andNutrition Research* **46**(2): 51- 57.

Kunele, E. J. (1999). Assessment of bacteriocin producting *Lactobacillus* Strain in the control of cereal based African ferment food. *Journal of Food Microbiology* **33**(42): 313-319.

Laca, A., Mousia, Z., Webb, C. and Pandiella, S. S. (2006). Distribution of microbial contamination within cereal grains. *Journal of Food Engineering* **72**(4):332-338.

Larone, D. H . (1998). *Medically Important Fungi: A Guide to Identification,* 3rd Edition, ASM Press, Washington DC, pp 205-209.

Los antiguos peruanos comían palomitas de maíz" . BBC Mundo. BBC. January 19, 2012.

Magan, N. and Evans, P. (2000). Volatiles in grain as an indicator of fungal spoilage, odour descriptors for classifying spoiled grain and the potential for early detection using electronic nose technology: A review. *Journal of Stored Product Protection* **36**: 319-340.

Marin, S., Sanchis, V. and Magan, N. (1999). Water activity temperature and ph effects on growth of *Fusarium*. *Journal of Moniliforme and Proliferation* **41** (3): 1063.

Marsh, S. F. and Payne, G. A. (1998). The colonization of sent corn by *Aspergillus Flanus*. *Journal of Phytopaology* **74**(1): 557.

Mathew, S., Thomas, G., and Ahmad, T. (2010). An evaluation on the impact of fungi on the 114 post-harvested Stored Wheat Grains. *International Journal of Biotechnology and Biochemistry* **6**(6): 34-45.

Matsuoka, Y., Vigouroux, Y. and Goodman, M. M. (2002). A single domestication for maize shown by multilocus microsatellite genotyping .*Proceedings of the National Academy of Sciences* **99** (9): 6080–6084.

Merriam-Webster Dictionary, definition 3 accessed June 7, 2012

Miller, J. D. (1995). Fungi and mycotoxins in grain: implications for stored product research. *Journal of Stored Products Research* **31**(1), 1-16.

Muir, W. and White, N. (2000). Microorganisms in stored grain. *Manitoba: Grain Preservation Biosystems* p 1-17.

Odunfa, S. A. and Adeyele, S. (2000). Preliminary study of the effect of lactic fermentation on the rheology and p^{H} of ogi porridge. *Journal of Food Microbiology* **20**(25): 71-75.

Omume, I. and Adeosun, A. (2010). A study of interaction of cultural and biological factors. Ph.D Thesis, London School of Hygiene and Tropical Medicine, London. pp150-155.

Osho, A., Mabekoje, O. O. and Bello, O. O. (2010). Comparative study on the microbial load of Gari, Elubo-isuand Iru in Nigeria. *African Journal of Food Science* **4**(10): 646 – 649.

Osungbaro, D. K. (2009). *Lactobacillus* in human health with reference to locally fermented foods. *Journal of Tropical Medicine* **2**(4): 28-36.

Ottogalli, G. and Galli, A. (1979). Microbiological quality of flours: sour dough for bakery products and spaghetti. In: *Food Microbiology and Technology. Proceedings of the International Meeting on Food Microbiology and Technology. Tabiabo (Parma) Italie* 20: 141-153.

Ozoh, K. E. and Kuyanbana, I. J. (2006). Sources of contamination of cereal crops. *Journal of Tropical Medicine* **12**(15): 21-23.

Pagán, J, Jaime R., Guachamín-Tello, A. M., Romero-Bastidas, M. E., Constantine-Castro, A. R. (2015). Late ninth millennium B.P. use of *Zea mays L*. at Cubilán area, highland Ecuador, revealed by ancient starches. *Quaternary International* **404** : 137–155.

Paolesse, R., Alimelli, A., Martinelli, E., Di Natale, C., D'Amico, A., D'Egidio, M. G. and Fanelli, C. (2006). Detection of fungal contamination of cereal grain samples by an electronic nose. *Sensors and Actuators B: Chemical* **119**(2): 425-430.

Pérez, A. E. and Erra, G. (2011). Identifying maize residues in pottery vessels in northwestern Patagonia, Argentina. *Magallania* **39** (2): 309–316.

Piperno, J. D. and Dolores R. (2011). The origins of plant cultivation and domestication in the new world tropics: patterns, process, and new developments. *Current Anthropology* **52** (4): 453–S470.

Rhodes, L. L. and Eagles, H. A. (1984). Origins of maize in new zealand. *New Zealand Journal of Agricultural Research* **27** (2): 151–156.

Richter, K. S., Dorneanu, E., Eskridge, K. M. and Rao, C. S. (1993). Microbiological quality of flours. *Cereal Food World* **38:** 367–369.

Roney and John (2009). The beginnings of maize agriculture. *Archaeology Southwest* **23** (1): 4.

Spicher, G. (1986). Merkpunkte für die Beurteilung der mikrobiologisch-hygienischen Qualitat von Weisenmehlen. *Die Mühle and Mischfuttertechnik* **33** : 449.

Spielvogel, A. G. and Jackson, J. (2005). Medieval and Early Modern Times: Discovering our past . Glencoe/McGraw-Hill School Publishing Company.

Tefera, T., Mugo, S., Beyene, Y., Karaya, H. and Tende, R. (2011). Grain yield, stem borer and disease resistance of new maize hybrids in Kenya. *African Journal of Biotechnology* **10**(23): 4777-4783.

Torrejón, F., Bizama, F., Araneda, A., Aguayo, M., Bertrand, S. and Urrutia, R. (2013). Deciphering the environmental history of the Aysén archipelagos, Chile: Colonial

influence and commercial exploitation during the Republican Era (XVI-XIX centuries). *Magallania* **41** (1): 29–52.

Wu, F., Bhatnagar, D., Bui-Klimke, T., Carbone, I., Hellmich, R., Munkvold, G. and Takle, E. (2011). Climate change impacts on mycotoxin risks in US maize. *World Mycotoxin Journal* **4**(1): 79-93.

Wu, F., Bhatnagar, D., Bui-Klimke, T., Carbone, I., Hellmich, R., Munkvold, G. and Takle, E. (2011). Climate change impacts on mycotoxin risks in US maize. *World Mycotoxin Journal* **4**(1): 79-93.

Akinrele, I.A. (1970). Fermentation studies on maize during the preparation of a traditional African Starch-cake food. *Journal of the Science of Agriculture*. **21**(12):619-625.

Adeniji, A.O. and Potter, N.N. (1978). Properties of ogi powders made from normal fortified and opaque corn. *Journal of Food Science* **43**:1571.

Akinrele, I.A. and Bassir, O.J. (1967). The nutritive value of ogi, a Nigerian infant food. *Journal of Tropical Medicine and Hygiene*. **70:**279.

Oranusi, S.U., Galadima, M., Umoh, V.J. and Nwanze, P.I. (2007). Food safety evaluation in boarding schools in Zaria, Nigeria using the HACCP system. Scientist Research Essay, **2**:426-433.