KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI

Evaluation of Filamentous Fungi in Selected Processed Indigenous Flours sold in the Kumasi Metropolis

BY

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DEDICATION

I dedicate this thesis to my dearest mother Rose Osew and father Thomas Awaitey and all my siblings for their prayers and support towards the successful completion of my studies. I also dedicate this work to Anthony Tobias Klein for his kind support and finally to Millicent Arhin for her inspiration. God richly bless you all.

DECLARATION

I hereby declare that the experimental work described in this thesis was performed by me at the Microbiology Laboratory, CAnLab, Kwame Nkrumah University of Science and Technology, Kumasi, under the supervision of Dr. F. C Mills-Robertson towards the MSc (Food Science and Technology) degree. I certify that, to the best of my knowledge, this work has not been submitted for the award of any other degree of the University, except where due acknowledgement has been made in text

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ABSTRACT

This study evaluated the filamentous fungi present in selected locally processed indigenous flour sold in the Kumasi Metropolis of Ghana using standard microbiological methods. Results from this study showed that indigenous processed flour was contaminated with different kinds of microorganisms exceeding the tolerable level. Dry cassava (kokonte) flour recorded mould count ranging from $1.70 \times 10^3 \pm 0.15$ cfu/g to $4.03 \times 10^5 \pm 0.35$ cfu/g while maize flour obtained mould count ranging from no counts to $1.18 \times 10^6 \pm 0.18$ cfu/g. Total plate count showed contamination levels between no counts to $9.1 \times 10^6 \pm 0.25$ cfu/g for the maize flour samples, while for the dry cassava (kokonte) flour counts ranged from $7.8 \times 10^3 \pm 0.30$ cfu/g to $4.64 \times 10^6 \pm 3.18$ cfu/g. Moisture analysis revealed percentage moisture content of 12.4%± 0.15 to 19.7% ± 0.12 for the maize flour samples and 10.9% ± 0.27 to 16.9% ± 0.56 for dry cassava (kokonte) flour. Coliforms test indicated negative for seven of eight (7/8) maize flour samples and six out of eight (6/8) for dry cassava (kokonte) flour samples bought from the various markets. From the study, thirteen filamentous fungi belonging to five genera were isolated from the various flour samples. Ten different species were isolated from the dry cassava (kokonte) flour while all thirteen were isolated from the maize flour. The isolated moulds species included; *Mucor racemosis*, *Cladosporium cladosporioides*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus wentii*, *Aspergillus ochraceus*, *Aspergillus versicolor*, *Cladosporium herbarum*, *Penicillium crustosum*, *Penicillium camemberti*, *Rhizopus stolonifer*, *Penicillium viridicatum* and *Mucor hiemalis*. The most prevalent species in the dry cassava (kokonte) flour was *Aspergillus flavus* occurring in about 77.8% of the samples while for the maize flour samples *Penicillium crustosum* was the most dominant species occurring in 44.4% of the samples. Information from questionnaires revealed that the source of the contamination may be due to the raw materials used in the flour production and also poor hygienic practices along the production chain.

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CHAPTER ONE

1.0. INTRODUCTION

The high incidence of post-harvest food losses, arising mainly due to inadequate food preservation technologies, is a major issue affecting the quality of food in West Africa, where seasonal food shortages and diseases resulting from nutritional deficiency are still a major concern (Aworh, 2008). Study has shown that fruits, vegetables, roots and tubers contribute to nearly 50% of perishable food commodities while grains such as maize, sorghum, millet, rice and cowpeas contribute to about 30% of food loss after harvest in West Africa (Aworh, 2008). Factors that contribute to these losses may include; inappropriate food processing technologies, poor harvesting and inefficient post-harvest handling practices, bad roads, moribund rail systems and many others (Aworh, 2008).

In Ghana, issues of post-harvest losses are predominant especially where locally produced crops such as cassava, yam, maize, rice, beans, and others are hardly processed leading to waste of crops especially during bumper harvest. In order to extend the shelf-life of some of these crops and hence reduce the incidence of postharvest losses, they are processed into flours and other products which may be used by individuals at home or sold on commercial basis. Methods involved in processing these indigenous foodstuffs may, however, expose them to contamination by several pathogens mainly fungi and some bacteria in addition to contamination from the farm before processing. For instance, most cereal grains can be contaminated by different species of microscopic fungus during it developmental stage (Scudamore, 2005) and these pathogens may affect the crop resulting in a reduction of the quality of the grain.

Some species may produce mycotoxins that intoxicate both humans and animal upon consumption (Scudamore, 2005). For instance, mycotoxin classes known to occur in cereals, including the

aflatoxins (AFB1, AFB2 and AFG1, G2), tricotecens, deoxinivalenol (DON) and (T-2 toxin), the fumonisins (FB1, FB2 and FB3), the zearalenone (ZON), ochratoxin A (OTA) and the ergot alkaloids ((Egbuta *et al*., 2014) are known to be carcinogenic. Studies have revealed that majority of these mycotoxins are produced by *Aspergillus*, *Penicillium* and *Fusarium* (Egbuta *et al*., 2014). Spores produced by these fungi are very difficult to eliminate due to their stability in high temperature and other harsh environmental conditions, hence the presence of these spores in food poses threat to the health of consumers. In the case of flour, the high grade types are treated to contain very low or no contamination due to use of advance technologies (Doolotkeldieva, 2010). Locally processed indigenous flours may, however, be contaminated by different microbes due to improper food safety practices and as these flours are usually sold on commercial basis, they may result in exposing consumers to several health risks.

The purpose of this study, therefore, was to evaluate the filamentous fungi of some selected processed indigenous flours sold in the Kumasi Metropolis.

1.1 PROBLEM STATEMENT

Different foodstuffs are processed into flour to be used for other food commodities and also to reduce postharvest losses and increase their shelf-life. Flour is an inevitable part of our diet which is used for many purposes; however, most of these flours get contaminated with different microbes including filamentous fungi and due to the spores produced by most of these organisms it is difficult to eliminate them from food. For instance, a study carried out by Doolotkeldieva (2010) revealed the presence of 27 types of fungi belonging to 7 genera in wheat flour samples collected from eight sites of flour manufactures in Turkey. Studies have also shown that filamentous fungi such as *Aspergillus*, *Penicillium*, *Fusarium*, *Alternaria*, *Acremonium*,

Cladosporium and *Curvularia* are micro-organisms commonly found in our surroundings, (Gołofit-Szymczak and Górny, 2010) probably because of their ubiquitous nature and also their ability to grow on any substrate when conditions are favourable (Klein and Paschke, 2004). These filamentous fungi are also known to produce mycotoxins in foods which can cause severe health implications upon exposure to consumers (Egbuta *et al*., 2014). Aflatoxin B1 produced by *A. flavus* and *A. parasiticus* has been classified by the International Agency for Research on Cancer (IARC) as a Group 1 carcinogen and has been reported as one of the aetiologies of liver cancer (Egbuta *et al*., 2014).

Though lots of studies have been carried out on industrially processed flours, information on microbial contamination of processed indigenous flour is limited, hence the need to conduct this present study that evaluated the microbial quality of some selected processed indigenous flour with respect to filamentous fungi in the Kumasi Metropolis.

1.2 JUSTIFICATION

The outcome of this study would bring to notice the different types of filamentous fungi contaminating locally processed indigenous flour. This would also inform the public/populace about the various types of filamentous fungi that are most common in the selected flours. The outcome of this result would also bring to bare the safety level of processed indigenous flour consumed by individuals in this country and comparing the experimental results with the accepted tolerable level of these microorganisms would bring to notice if these processed flours are safe for consumption.

If the results of this study prove to show that the processed flour are not safe due to contamination then measures could be taken to ensure that the contamination level is reduced or minimized to an

acceptable level. If on the other hand, the results show that the processed flour are safe then this would increase the confidence of local manufactures.

1.3 MAIN OBJECTIVE

To evaluate the presence of filamentous fungi in some selected locally processed indigenous flours sold in the Kumasi Metropolis.

1.4 SPECIFIC OBJECTIVES

1. To determine the moisture content of the locally processed indigenous flours.
2. To determine the load and type of filamentous fungi in the selected locally processed indigenous flours.
3. To determine the total plate count of mesophilic microbes and coliforms present in the selected locally processed indigenous flours.

CHAPTER TWO

2.0. LITERATURE REVIEW

2.1. POSTHARVEST LOSSES

The Food and Agriculture Organization of the United Nations, has predicted a global food loss of about 1.3 billion tons every year (Gustavsson *et al*., 2011). For instance, it is estimated that about 1.6 million tons of food are wasted in the United Kingdom because they do not meet the retailer standards (FAO, 2013) with an estimated 6.7 million tons of food going waste each year. In the case of the United States, food loss contributes to about 30% of all food produced (FAO, 2013).

It has been estimated that the world‟s population is likely to shoot up to 10.5 billion by 2050 (Aulakh and Regmi, 2013), thus, further posing concerns to the global food security issues. In order to address these concerns, it is estimated that food supply would have to be increased by about 60% (estimated at 2005 food production levels) (Alexandratos and Bruinsma, 2012) in order to feed the extra mouths. Thus, it is critical to increase production while reducing food losses, however, the issue of postharvest losses is another challenge to deal with.

This challenge is also significant in developing countries where food losses in the Sub-Saharan Africa are estimated to be about $4 billion per annum, estimated to feed about 48 million people (FAO, 2013). Losses on cereals are estimated to be high, accounting for about 25% of the total crop harvested. These losses can even be higher for perishable products accounting for as high as 50% of harvested fruits, vegetables and root crops (Voices Newsletter, 2006).

In order to reduce postharvest losses as a result of raw material spoilage and hence increase food availability, most raw materials are processed into different products that are expected to extend the shelf life of the product and a typical example of such products is flour.

2.2. FLOUR

Flour is a powder, made by grinding or blending cereal grains, beans, or other seeds or roots such as cassava, potato among many others. It serves as the main ingredient for bread making, biscuits, cake or pastries and may also serve as a staple food for many cultures (Vaclavik and Christian, 2008).

There are many different varieties of flour available on the market including wheat flour, banana flour, cassava flour, yam flour, peanut flour, maize flour and many more with wheat flour being the most common type across the world (Vaclavik and Christian, 2008). Flour may be produced by means of industrial processing or traditional processing, but the focus of this study is on traditionally processed flour due to the unstandardized approach involved in processing it hence the likelihood of high contamination.

Traditional foods and their processing techniques form part of the culture of indigenous people and these techniques constitute a vital body of indigenous knowledge passed on from one generation to another (Aworh, 2008). Indigenous foods contribute to great portion of the agroindustries which account for more than 50% of value-added manufacturing, exports and employment in most African countries including Ghana (Adu-Gyamfi and Appiah, 2012).

Different foodstuffs are processed into flour for different reasons either to extend its shelf life or to be used for preparation of different products. In most traditional settings, different approaches (non-standardized methods) are employed in the processing of flour thereby exposing the flour to contamination by microorganisms. This makes processed indigenous flours microbiologically unsafe for consumption as compared to the industrially processed flour.

It has been observed that traditionally processed foods are well-preserved through biochemical activities which lower the pH and prevent growth of pathogenic microbes (Adu-Gyamfi and

Appiah, 2012). For instance, research has indicated that the microflora of most fermented foods are dominated by lactic acid bacteria, which inhibit the growth of foodborne pathogens such as *Salmonella* spp., *Shigella* spp., *Escherichia coli,* and *Staphylococcus aureus* in some foods (Jesperson *et al*., 1994).

In spite of these inherent preservative properties offered by fermented foods, the poor processing procedures result in end products of poor quality. It has been noted that maize and cassava products are often contaminated as a result of deficiencies in processing methods, such as insufficient drying, and poor packaging. Open sun drying of such foods result in contamination by various microorganisms (Jesperson *et al*., 1994).

With the current rising trend of food safety issues across the world, this study seeks to evaluate the mould contamination in processed indigenous flour (dry cassava „kokonte" flour and maize flour) sold in selected markets in the Kumasi Metropolis.

2.2.1. CASSAVA FLOUR ('KOKONTE' POWDER)

Cassava (*Manihot esculenta*) serves as one of the most important staple drought resistant crops produced in Ghana. It is estimated that cassava provides about 40% of the calories consumed in Africa and serves as the most valuable source of energy to developing countries (Sanful and Darko, 2010). According to Falade and Akingbala (2010), cassava contains about 70% carbohydrate and hence considered the fourth most energy-rich food. Study has indicated that the consumption of cassava products can help to support the nervous system and relieve stress, anxiety and irritable bowel syndrome (Baffour, 2009) and this has been accredited to the high carbohydrate content. It has been established that Africa contributes to about 54% of global cassava production annually

and this is an indication that cassava is of high value in Africa (Uchechukwu-agua and Caleb, 2015).

Plate 1: Cassava root

Despite the importance of cassava to Africans, it is among the many crops which deteriorate quickly after harvest owing to its high moisture content (60 to 75%) (Sánchez *et al.*, 2010). Other factors that may contribute to the quick deterioration of cassava include; pest, diseases and microbial attack to exposed areas caused by mechanical damage (Iyer *et al.*, 2010). This situation confers a limitation on the shelflife as well as the utilization of cassava root and hence the processing of cassava into other food forms, such as flour, to enhance stability and extend the shelf life (Uchechukwu-agua and Caleb, 2015).

„Kokonte' powder is a type of flour made from dried cassava root. This flour is used in the preparation of „face the wall" or „kokonte"; a staple food in Ghana which is usually accompanied with groundnut soup. The process of making this flour involves washing of the unpeeled cassava, peeling, washing, cutting, drying, milling into flour, and sieving (Sanful and Darko, 2010).

Indigenous products such as „kokonte' contribute significantly to food security situation in the country because of their availability, affordability, nutritional quality and inherent preservative properties (Adu-Gyamfi and Appiah, 2012). In spite of the inherent preservative properties of this flour, there are several issues surrounding the safeness of „kokonte" flour. Mycological studies have confirmed the presence of several toxigenic fungi and mycotoxins in foods including „kokonte" (Wareing, 1993). These findings as reported have serious health effects since some mycotoxins are carcinogenic, mutagenic and teratogenic (Guengerich *et al.,* 1996).

Plate 2: Kokonte flour

2.2.2. MAIZE FLOUR

Maize (*Zea mays*) serves as one of the major staple foods in the developing world, mostly in the Sub-Saharan Africa. It is cultivated in over 70% of total arable land in Malawi and contributes significantly to the diet of more than 80% of the population (Matumba *et al*., 2009). Maize is one of the cheapest commodities found on the Ghanaian market. It serves as a domesticated food item and many regions in the country virtually have it in their cuisines (Aliyah, 2014). It is estimated that over 85% of maize produced in Ghana is for the purpose of human consumption while the remaining 15% is for animal feed (Rondon and Ashitey, 2012).

Maize can be enjoyed in various forms such as corn gluten, whole corn, corn meal, corn flour, corn starch, and many others. It has been estimated that an average of 1.5 million metric tonnes of maize was produced between 2007 and 2010 accounting for about 62% of total grain output in the country (Rondon and Ashitey, 2012).

Maize among other cereals is processed into flour not particularly for the purpose of extending its shelflife but rather due to the diverse use of maize in its flour or dough form. Though the flour can be used in preparation of different food products, porridge is the most notable cuisine of maize flour. It may also be used together with cassava flour in the preparation of „*tuozafi'* a delicacy among the people of the northern part of Ghana.

The traditional method of preparing maize flour involves dehulling, steeping, washing, milling, drying and further milling into fine powder (Gwirtz and Garcia-Casal, 2014). This method may differ slightly from one community to the other depending on what the flour would be used for. The method of processing maize into flour has raised several concerns with regards to food safety since food safety measures are not practiced by the producers. This has been confirmed by several research works carried out in different countries (Atanda *et al*., 2013).

Studies have shown that grains are generally vulnerable to attack by mycotoxigenic fungi which includes *Aspergillus, Fusarium* and *Penicillium* (Moreno *et al.*, 2009). The occurrence of these organisms in maize flour has been confirmed by Alborch *et al.*, (2012) when they conducted a study on 30 maize flour samples of which 14 were found to be contaminated by moulds resulting in the production of Aflatoxin A. Thus, the presence of these organisms may not only affect the nutritional and quality of the grains but may also lead to the production of mycotoxins in the products. It is estimated that about $225 million per year, out of the $932 million value of maize is lost to aflatoxin in the United States alone due to fungal contamination (Betran and Isakeit, 2003).

In developing countries, the incidence of maize flour contamination by fungi has been reported by several researchers. For instance, studies by Matumba *et al*., (2009) showed the presence of moulds in maize flour resulting in the production of mycotoxins. The trends of research with regard to these organisms and their mycotoxins have been diverted towards peanuts giving flours little attention (Matumba *et al*., 2009). This incidence poses a great threat to the food safety issue as well as food security worldwide.

2.3. FOOD SAFETY

The purpose of food is to sustain and support life but unfortunately this is not always the case since sometimes food may contain chemical, biological or physical hazards which may pose severe threat to the health of consumers. It therefore becomes paramount to put measures in place to ensure that food is safe for consumption.

In every part of the world, efforts are being made to combat against food contamination, foodborne diseases and food wastage (Käferstein and Moy, 2003). These efforts began in prehistoric times, where different cooking methods were first employed. Today, more sophisticated technologies are being used in food preservation and to make food safer, however, despite these advances in technology, the safety of food is still an issue which calls for concerns (Käferstein and Moy, 2003). In regard to issues surrounding food safety, many studies have shown that there is a worldwide increase in the occurrence of foodborne disease, and is a substantial cause of death nationwide (Scott, 2003). According to Clarence *et al*., (2009), foodborne diseases are diseases resulting from ingestion of microorganisms, toxins and cells produced by the microorganisms present in food. The intensity of the signs and symptoms may vary depending on the amount of contaminated food ingested by individuals. Diseases resulting from foodborne pathogens are severe in victims with

compromised immune systems (Clarence *et al*., 2009). The issue of food security is a complex one in both developed and developing countries, where nutrient source from meat and meat products, fish and fish products, cereals and grains among others are generally regarded as high risk and unwholesome commodities with respect to pathogen contents, availability of natural toxins and other possible contaminants (Antwi-Agyei and Maalekuu, 2014).

Foodborne microbiological hazards present an important challenge to food safety experts worldwide each year as it may be responsible for many reported cases of illness and death (Batz *et al*., 2005). To reduce the incidence of foodborne disease, many experts and stakeholders urge the development of a science- and risk-based food safety system, in which decision makers prioritize hazards and interventions using the best available data on the distribution and reduction of risks (Batz *et al*., 2005). Such a system requires an understanding of the many risk factors between the point of production and the point of consumption and the ability to systematically target intervention efforts along this "farm-to-fork" continuum (Batz *et al*., 2005).

The issue of food safety is not only a problem in developing countries but also in developed countries, where advanced food chain monitoring systems have been put in place (Scallan *et al*., 2011). Thus, food-borne diseases are still a major challenge worldwide despite the important efforts being put in place to reduce the occurrence of certain pathogens through better farm practices and food regulations (Scott, 2003).

2.3.1. FOOD SAFETY ISSUES IN DEVELOPED COUNTRIES

Foodborne infections are a major health problem worldwide which results in economic reduction. It is a major cause of illness and death globally and recognizing this, the World Health Organization (WHO) developed its Global Strategy for Food Safety (Adak *et al.*, 2005). In the industrialized world, incidence of foodborne diseases is known to cause considerable illness which affects the

healthcare systems (Adak *et al.*, 2005). It has been estimated that up to about one-third of industrialized countries in general suffer from foodborne illness each year. Global report indicates that approximately 2.2 million deaths caused by diarrhoea are recorded annually worldwide and most of these cases are ascribed to contaminated food and water (Saba and Gonzalez-zorn, 2012). Although most of the cases are mild, a considerable number are fatal and a high incidence of acute infections and chronic sequelae can lead to billions of dollars in medical costs, loss of productivity, and frequent recalls (Saba and Gonzalez-zorn, 2012). Studies have shown, for instance, that in developed countries, food pathogens are responsible for millions of cases of gastrointestinal infectious diseases each year, costing billions of dollars in medical care and loss of productivity (Saba and Gonzalez-zorn, 2012). In the United States alone, it has been estimated that about 46 million foodborne infections occur each year, along with 250,000 hospitalizations and 3,000 deaths (Scallan *et al*., 2011). Ternhag *et al*. (2008), reported that gastrointestinal infections result in illness and death which contribute to economic loss in most parts of the world, including advance countries that have developed surveillance and control programs.

2.3.2. FOOD SAFETY ISSUES IN DEVELOPING COUNTRIES

Reports have shown that foodborne diseases are the major causes of illness and death in the developing world, accounting for the loss of about 1.8 million people annually (Scallan *et al*., 2011). The incidence of foodborne diseases in developing countries is assumed to be higher than reported since most incidences which occur at homes are not reported. Foodborne infection causes death in many children and the resulting diarrhoeal disease can have lasting effects on children's growth and development (Adak *et al*., 2005). Report from Nigeria shows that several food items have been implicated with high incidence of bacterial contamination (Clarence *et al*.,

2009). The Food and Agricultural Organization (FAO) of the United Nations and the World Health Organization (WHO) stated that diseases that result from food contamination is possibly the most prevalent health problem and an important cause of loss in economic productivity (Käferstein and Moy, 2003).

In Ghana, it is estimated that, about 84,000 deaths were caused by diarrhoea from 2004 to 2008 accounting for 25 percent of deaths among infants under five years of age (Tuffuor, 2008). While it is considered that most cases of diarrhoea in less developed countries are waterborne related, Scott (2003) stated that, contaminated foods also have a part to play and that there is an urgent need to integrate food safety, along with water and sanitation programs, as an essential strategy to curb the disease.

The Food and Drugs Authority (FDA) in Ghana, report of 2006 indicated that 90,692 people died from food and personal hygiene-related illnesses in the country, during which an estimated 297,104 patients were reported at the various Out-Patients Departments of clinics and hospitals with similar cases (Tuffuor, 2008). The report also indicated that the treatment of these diseases alone in 2006 cost the government GH¢594,208.00. Twelve victims including a medical doctor and three student nurses were reported dead due to cholera outbreak in Cape Coast, the Central Regional capital of Ghana and a total of 922 cases were reported in various health facilities in the metropolis (Asiedu-Addo, 2014).

It is unpleasant to know that in developing countries, significant proportion of the annual budget of both government and development partners is spent confronting food-borne diseases (Tuffuor, 2008) and therefore it is necessary to recognize and address food safety issues. Minimizing the consumption of unsafe food, therefore, may help ensure the good health of individuals and hence, contribute to economic growth in developing countries.

2.3.3. SAFETY ISSUES ON FLOUR

In Africa and other parts of the world, processing of cereals, tubers and fruits into flour has been an integral part of food preservation culture for ages now (Jayeola and Akinsebikan, 2013). One reason for processing food into flour is to ensure safeness and also extend shelflife due to the low moisture content of such a product; however, studies have shown that there are still issues surrounding the safeness of flour.

It is estimated that every year, one out of six Americans gets infected with foodborne disease, resulting in approximately 128,000 hospitalizations and 3,000 deaths, and these have been attributed to *Salmonella* and *E. coli* O157:H7, two microorganisms that have been recognized as the agents of foodborne outbreaks worldwide (CDC, 2011). Although these organisms may be present in a variety of raw materials, many are unaware that flour is one of the most common ingredients with high prevalence of food pathogens (Akins, 2011).

Foods and other ingredients with low-moisture content have not traditionally been considered for discussion in terms of food safety, because these products do not offer favourable environments for growth of microorganism, however, some organisms such as *salmonella*, moulds and others have been associated in several foodborne outbreaks in low-moisture foods (Dack, 1961).

Flour has also been implicated with foodborne outbreaks although it is considered safe due to the validated kill step involved in production, such as baking or cooking (Dack, 1961). For instance, in 1952, flour was suspected to be the cause of an outbreak of *Salmonella paratyphi* B phage type 1 which occurred in New South Wales, Australia, although the causative organism was not isolated from the sample (Dack, 1961). In 2008, *Salmonella* outbreak in New Zealand was also implicated to flour in which sixty-six cases of illness were reported, of which eight patients were hospitalized

(Eglezos, 2010). There was not much evidence, but investigations pointed out that victims (especially children) were more likely to have consumed uncooked flour in homemade play dough, and raw cake and batter mixes (Eglezos, 2010).

Other pathogens such as *E. coli* O157:H7 have also been implicated with diseases outbreaks in flour-containing products. In 2009, prepackaged, ready-to-bake cookie dough was recalled due to *E. coli* O157:H7. Seventy seven (77) consumers of the unbaked dough became ill while thirty five patients were hospitalized with ten (10) developing life-threatening haemolytic uremic syndrome (O‟Neil *et al*., 2011).

2.3.4. MICROBIAL CONTAMINATION OF FLOUR

Flour has low moisture content with water activity (a_w) of about 0.85. Due to this, there is the misconception that microorganisms cannot contaminate flour, however, this misconception has been proven wrong by several research works (Doolotkeldieva, 2010; Bullerman and Bianchini, 2014). In a study carried out by Doolotkeldieva, (2010), twenty seven different species of fungi belonging to seven genera were found to contaminate flour samples of different grades including first and high grade flours. This is an indication that flours produced may not be wholly safe for consumption. Studies have shown that most cereal products (such as flour) are contaminated by different organisms such as yeasts, moulds, bacteria (psychrotrophic, mesophilic, and thermophilic/thermoduric) (Bullerman and Bianchini, 2014). *Bacillus cereus*, *Salmonella*, *Clostridium botulinum*, *Escherichia coli*, *Clostridium perfringens*, and *Staphylococcus aureus* are also known bacterial pathogens that cause contamination to cereal grains and their products thereby causing problems to consumers (Bullerman and Bianchini, 2014).

Though different microorganisms may contaminate flour samples, the focus of this study is on filamentous fungi in locally processed indigenous flour of cassava flour („kokonte") and maize flour since there is limited information in Ghana.

2.4. FILAMENTOUS FUNGI

Filamentous fungi (moulds) belong to a large group of fungal species characterized by discolouration and a fuzzy appearance, especially on food (Morgan, 1999). These organisms are multicellular and contain spores which spread in air to begin the growth of new moulds. Under favourable conditions, these organisms grow producing a fluffy growth, either white or gray, but also depending on the type of mould may appear red, bluish green, orange or some other colour (Morgan, 1999). Most filamentous fungi (moulds) are mesophiles growing between a temperature range of 25° and 30°C in warm damp places. Some are psychrophiles and are able to grow in refrigerated temperature while others are thermophiles growing at high temperature with very few being opportunistic pathogens of human (Kilikian, *et al*., 2014).

Studies have shown that filamentous fungi such as *Aspergillus*, *Penicillium*, *Fusarium*, *Alternaria*, *Acremonium*, *Cladosporium* and *Curvularia* are common food contaminating microorganisms that may be inhabitants of the soil, air or other parts of the environment (Gołofit-szymczak, 2010). The ubiquitous nature of these organisms enables them to grow and survive on any substrate when conditions are favourable (Klein and Paschke, 2004). These organisms derive energy from the organic substrate on which they thrive, by means of heterotrophy. These organisms secrete hydrolytic enzymes from their hyphal tips which degrade complex biopolymers such as starch, cellulose and lignin into simpler forms which can be absorbed by the hyphae. In this way,

the organisms are able to bring about decomposition of organic material and recycling of nutrients throughout the ecosystems (Lindahl *et al.*, 2007).

Moulds are able to thrive in environments where conditions are unfavourable to other organisms. For instance, they are able to survive at pH too low for most bacterial activities hence able to cause food spoilage at conditions unfavourable to bacteria (Marriott, 2012). Organic acids, which the bacteria in general cannot tolerate, may also be metabolized by moulds as a source of energy, and these acids may be oxidized to carbon dioxide and water (Marriott, 2012). Moulds can even survive under conditions with high osmotic pressure as seen on the surface of jellies and jams which have high sugar content (Fung, 2009). They can also grow on diverse array of foods, from foods with high acidic content such as lemon to those with neutral pH such as bread and other starchy foods.

A small proportion of moulds found on food stuffs is capable of producing mycotoxins (Vaclavikova *et al.*, 2013) and the best known of these are the aflatoxins produced by moulds growing on peanuts, wheat and millet which were not dried as soon as they were harvested (Mutegi *et al.*, 2013).

2.4.1. CONTAMINATION OF FLOUR BY FILAMENTOUS FUNGI

Though research has shown that microorganisms are unable to survive in food commodities with low water activity, several studies have confirmed the presence of moulds in flours. In a microbiological survey conducted on milled cereal grains (from 2003 to 2005) using routine data from North American dry-milling operations, different organisms including moulds were found to contaminate wheat flour, with the average levels of microbiological populations of mould being 2.58 log CFU/g (Sperber, 2007).

In a study carried out in Argentina on the mycoflora of different wheat products which includes wheat flour, the results obtained indicated the presence of different filamentous fungi of the genus *Cladosporium, Alternaria, Aspergillus, Penicillium, Eurotium, Epicoccum, Fusarium* and

Rhizopus (Aringoli *et al.*, 2012). From the results they obtained, the count of mould in the flour was quite low because it had undergone several processing steps, however according to Kozak *et al*. (1979) as cited by Aringoli *et al*. (2012), the detected level based on viable count method was high enough to cause breathing problem. At the end of their 90 day study, organisms of the genera *Aspergillus* and *Eurotium* were present during the first three days of study, while *Mucoraceae* family, which includes principally isolates of the genera *Rhizopus*, were the most abundant at day 90. In another study, there was contamination with aflatoxin-producing fungi and aflatoxin B_1, affecting 545 samples of wheat grains, 475 samples of intermediate products of wheat grain being milled to flour (like middlings), and 238 samples of flour (Halt, 1994). In the study, although *Aspergillus* (34.87%) and *Penicillium* (32.37%) dominated, other types such as *Cladosporium*, *Fusarium, Mucor, Alternaria, Rhizopus, Absidia* and *Trichoderma* were present.

In a study conducted to assess the microbial quality of the two different flours, that is, wheat flour collected from various households produced from traditional flour mill, and wheat flour purchased from retail shops, higher coliform and fungi counts were observed from house-made samples compared with the commercial samples (Ennadir *et al*., 2012). Bacteria isolated from the samples belonged to the genera: *Enterobacter* spp., *Serratia* spp., *Klebsiella* spp., *Pantoea* spp., *Leclercia* spp., *Proteus* spp. and the most predominant genera was *Aspergillus* (81 %). In a study by Aringoli *et al.*, (2012), the most predominant genera was rather *Rhizopus.* The microbial counts were; however, lower than the specifications laid down in the Codex Alimentarius, attributing to these flours as having satisfactory microbiological quality.

2.4.2. MYCOTOXINS CONTAMINATION BY FILAMENTOUS FUNGI

Filamentous fungi are also known to be producers of secondary metabolites (mycotoxins) which have been investigated to have adverse health implications on both humans and animals when exposed to them (Muri *et al.*, 2009). Aflatoxin B1 produced by *A. flavus* and *A. parasiticus* has been classified by the International Agency for Research on Cancer (IARC) as a Group 1 carcinogen and has been reported as one of the aetiologies of liver cancer (IARC, 2012). Thus, mycotoxins are abiotic hazards produced by some groups of fungi that can grow on a variety of crops (Marin *et al.*, 2013). Physicochemically, mycotoxins are thermostable and in most cases aromatic and nonantigenic low-molecular metabolites (Doolotkeldieva, 2010) and exert a diverse range of toxic evicts because their chemical structures are very heterogeneous.

Mycotoxins are major concern in grain storage, as they may be produced under previously existent conditions such as the moisture content, temperature, storage period, contamination rate, broken grain and impurities, insect presence, oxygen rate, damages during harvest processing and grain, and seed transport (Scudamore, 2005). Grains can be contaminated by a great variety of microscopic fungus during its development and these organisms may affect the plant thereby resulting in a reduction of the grain quality as well as products made from the grain (Scudamore, 2005).

The main mycotoxin classes that occur in cereal are the aflatoxins (AFB1, AFB2 and AFG1, G2), the tricotecens, deoxinivalenol (DON) and (T-2 toxin), the fumonisins (FB1, FB2 and FB3), the zearalenone (ZON), ochratoxin A (OTA) and the ergot alkaloids. The majority of the mycotoxins in these groups are produced by three fungi genus; *Aspergillus, Penicillium* and *Fusarium* (Birck *et al.*, 2005). Some fungus develop in different temperatures and produce toxins in really low temperatures such as the ones from the *Fusarium* spp. This is one of the reasons why in some

Brazilian regions, the winter crop grain have different toxins from those produced during the warm seasons (Birck *et al*., 2005).

In a study by Halt (1994), *Aspergillus flavus*, the known aflatoxin producer, was detected in 9.94% of analyzed samples while Aflatoxin B_1 was found in 76.8% of samples contaminated with *A. flavus*. The highest contaminations with aflatoxin B1 was detected in wheat grain samples (mean value of 16.3 µg/kg) and in intermediate products of wheat grain being milled to flour (mean value of 11.13 µg/kg). Contamination was lower in flour samples (mean value of 4.13 µg/kg). In accordance to proposed standards given by the FAO and WHO, under which the content of aflatoxin should not exceed 30 µg/kg in food products, only two of the 96 samples analyzed did not meet the set criteria (Halt, 1994).

2.5. COLIFORMS

Coliforms are indicator microorganisms living in the intestinal tracts of humans and animals hence are excreted through the faeces of these organisms (Leclerc *et al.*, 2001). The presence of coliforms in food material is an indication of faecal contamination and hence the possible occurrence of faecal pathogens such as *Salmonella* and S*higella* species (Chou *et al.*, 2004). Flour, due to their low moisture content, are regarded as safe against microorganisms such as coliforms since they are unable to survive under such condition, but studies have also shown the presence of these organisms in flour samples as indicated by Victor *et al.*, (2013). High level of coliforms in food may result in diarrhoea outbreak and other secondary complications (Sivaraja and Nagarajan, 2014).

CHAPTER THREE

3.0. MATERIALS AND METHODS

3.1. MATERIALS

3.1.1. Study Area

Kumasi is located in the transitional forest zone, about 270km north of Accra. It covers a total land area of 254km^2 and has an elevation ranging between 250 – 300 metres above sea level. The Metropolis is bounded to the north by Afigya Kwabre and Kwabre East Districts, to the west by Atwima Nwabiagya District to the east by Ejisu Juabeng and Bosomtwe-Atwima Kwanwoma Districts, and to the south by Atwima Kwanwoma District (Attuquayefio and Abdulai, 2013).

The Metropolis has a population of 2,035,064 representing 42.6% of the total regional population (Ghana Statistical Service, 2012).

In selecting the study area, the Metropolis was divided into four areas and a market was selected from each part. The Central Market, Bantama Market, Atonso Market and Ayigya Market were the selected markets for the study and they were selected in such a way to reflect both major markets (where Vendors from far and near come to sell) and community markets.

3.1.2. SAMPLE AND SAMPLE COLLECTION TECHNIQUE

Two types of processed indigenous flours were considered for this study; maize flour and cassava flour („kokonte' powder). These two flours were considered for the study because they are very common and frequently used in food preparation in the Kumasi Metropolis. Sixteen samples (eight of each flour) were selected for this study from the four different markets in the Metropolis. For each market, two Vendors were selected at random for sample collection and administration of questionnaires and from each Vendor three samples were bought to serve as replicates. Vendors

were not located at the same place so locations of second vendors were enquired from the first ones. Samples were packaged into sterile sample bags and brought to the laboratory for analysis. Samples were analyzed on the same day they were bought from the market, but those that were not analyzed same day were stored in the refrigerator at 4°C. Samples were analyzed in batches, four samples per batch. Two samples (maize flour and kokonte flour) were also processes in the laboratory to serve as controls.

3.1.3. CHEMICALS, MEDIA AND REAGENTS

Bacteriological peptone water (Oxoid LP0037), Potato Dextrose Agar (Roth Carl), Dichloran Rose Bengal Chlortetracycline (DRBC) agar (Fluka analytical 17147), Oxytetracycline Gentamycin Yeast Extract Glucose (OGY) agar (Oxoid CM0545), Plate Count Agar (Fluka analytical 70152) and MacConkey broth (CM0005) were obtained from the microbiology store at the CAnLab, Department of Biochemistry and Biotechnology, KNUST.

3.2. METHODS

3.2.1. Sterilization of Equipment and Materials

Materials used for this study were subjected to sterilization under laboratory conditions using standard procedures (Cheesbrough, 2009). Test tubes and glass petri dishes were washed thoroughly with soap and water, rinsed, air dried and sterilized in an oven at 170 °C for two hours. In order to prevent the media from getting contaminated, all media and inocula were prepared under aseptic condition within the inoculating hood with the ultra violet light turned on for about 30 minutes before use. Furthermore, open ends of all test tubes containing media and samples were sterilized using naked flame before and after inoculum transfer. The inoculation needles were

flamed until red hot before and after each inoculation. Culture media were also only opened when they were ready to be used and the work benches thoroughly cleaned with 70% alcohol before and after work.

3.2.2. Moisture Content Analysis

Two (2) gram sample was weighed into a petri dish and placed in an oven at 130°C for about 2 hours, weighing intermittently until there was no change in weight (University North Dakota State, 2014). The samples were cooled to room temperature in a desiccator at each time before weighing. The moisture content was express as;

(Weight loss / initial weight of flour) x 100%

3.2.3. Microbial Sample Preparation

Working under aseptic condition, ten grams (10 g) of each sample was weighed using a sterile weighing boat and transferred to sterile sample bottles containing 90 ml sterile peptone water (Egbuta *et al*., 2014). Each sample was vortexed for about 1 minute at moderate speed and serially diluted to make five dilutions (10^{-1} - 10^{-5}) by transferring 1 mL homogenized sample to 9 mL dilution blank, mixing well until the 10^{-5} dilution was obtained. Aliquots (0.1 mL) of these dilutions were used for the study.

3.2.4. Microbial Enumeration

Spread plate method of inoculation was employed in the microbial examination of the samples. From the prepared 10-fold serial dilutions, enumeration of moulds were carried out by the spread plate method on Potato Dextrose Agar containing 100 mg/L of chloramphenicol and 50 mg/L

Oxytetracycline to suppress the growth of bacteria (Egbuta *et al.*, 2014). The plates were incubated at 25 °C for 5 to 7 days. After the appropriate incubation periods, dilutions with 20200 colonies were selected and manually counted. The number of colony-forming units per gram (cfu/g) of samples was calculated by multiplying the number of organisms by the dilution factor.

3.2.5. Isolation and Identification of Moulds

Three different media; Potato Dextrose Agar (PDA), Oxytetracycline-Glucose Yeast Extract Agar (OGY) and Dichloran Rose Bengal Chlortetracycline Agar (DRBC) (each containing 100 mg/L of chloramphenicol) were used for the isolation of the moulds. From the prepared dilutions, 0.1 ml of the inoculum was inoculated onto the different media by the spread plate method and the plates incubated at 25 °C for 5 to 7 days.

3.2.6. Subculture of Moulds

Spores from different colonies were picked from the different media with the aid of a sterile inoculation needle, and sub-cultured onto the respective media using the three point method. The plates were then incubated at 25 °C for 5 to 7 days.

3.2.7. Identification of Moulds

Mould cultures were prepared by lifting the mycelia mat of the organism with a sterile inoculation pin into a drop of lactophenol blue on a slide, teased (spreading the mat) and covered with a cover-slip and observed under a microscope. Different characteristic features of the isolated organism were observed and used in their identification using the fourth edition of introduction to foodborne fungi (Robert *et al.*, 1995).

3.2.8. Total Plate Count

Spread plate method of inoculation was used to determine the total plate count of organisms in the samples. From the prepared serial dilutions, enumeration was carried out by the spread plate method on Plate Count Agar. The plates were incubated at 37 °C for 24 hours. After the appropriate incubation, plates with 30-300 colonies were selected and manually counted. The number of colony- forming units per gram (cfu/g) of samples was calculated by multiplying the number of organisms by the dilution factor.

3.2.9. Coliform Test

Sterile MacConkey broth was prepared in test tubes to carry out the coliform test. From the prepared serial dilution, 1 mL of inoculum from the 1:10 dilution (10^{-1}) was transferred into 9 mL of MacConkey broth under aseptic condition. Incubation was done at 37 °C for 24 hours and test tubes which showed change in media colour from red to yellow were recorded as positive.

3.3. STATISTICAL ANALYSIS

Analysis was done in triplicates in order to minimize the error margin as much as possible. Results obtained were tabulated into Microsoft Excel 2010 and for easy interpretation, the data was subjected to one way analysis of variance (ANOVA) and the significance differences between the means of the various markets determined by using Statistical Package for Social Sciences (SPSS version 20). P-values ≥ 0.05 were considered as statistically not significant.

CHAPTER FOUR

4.0. RESULTS

This chapter captured the outcome of the methods outlined in the previous chapter so as to achieve the aim and specific objectives of the study. Results obtained for the moisture analysis of the sixteen (16) samples and the controls have been tabulated below. Enumeration of the moulds, total plate count, coliform test as well as the identification of the various moulds isolated from all the samples were also presented.

4.1. MOISTURE CONTENT OF THE SAMPLE

The average moisture content of the maize flour samples ranged from 12.37%± 0.15 to 19.70% ± 0.12 (Table 1). Flour from Vendor-2 in the Central market (Cm MF2) recorded the highest moisture content while the control sample (CMF) recorded the lowest. Samples from Ayigya market (Aym) recorded 15.07% ± 0.19 and 13.33% ± 0.37 for Vendor-1 and Vendor-2 respectively, Atonso market (Atm) recorded 13.87% ± 0.12 for Vendor-1 and 13.10% ± 0.25 for Vendor-2. For Bantama market (Bm) 15.50% ± 0.15 was obtained for Vendor-2 and 13.23% ± 0.09 for Vendor-1. The moisture content for the cassava flour ranged from 10.93% ± 0.27 to 16.90% ± 0.56 as recorded by the Vendor-1 from Atonso market (Atm KF1) and the Vendor-2 from the Central market (Cm KF2) respectively. The control sample had a moisture content of 11.70% ± 0.17 while samples from Bantama market (Bm) had 15.00% ± 0.06 and 13.03% ± 0.15 for Vendor-1 and Vendor-2 respectively. For Ayigya market, 12.57% ± 0.34 and 13.03% ± 0.29 were recorded as moisture contents for samples from Vendor-1 and Vendor-2 respectively.

Table 1: Moisture content of maize flour from different Vendors

Sample code	Aym MF1	Aym MF2	Cm MF1	Cm MF2	Atm MF1	Atm MF2	Bm MF1	Bm MF2	CMF
Mean Moisture content/%	15.07± 0.19 ^{c}	13.33 ± 0.37 $_{a\,b}$	14.03± 0.18 ^{b}	19.70 ± 0.12^{d}	13.87 ± 0.12^{b}	13.10 ± 0.25$_{a\,b}$	13.23 ± 0.09$_{a\,b}$	15.50 ± 0.15^{c}	12.37± 0.15^{a}

The abbreviations indicate the markets, maize flour and vendors from whom the maize flours were bought.

Key: Aym - Ayigya market, Cm - Central market, Atm - Atonso market, Bm - Bantama market, CMF - Control Maize Flour, MF- maize flour, 1 and 2 – Vendors 1 and 2 from the same market. Means that do not share the same letter (superscript) were significantly different (P<0.05) but those that share the same letter (superscript) do not differ significantly (P>0.05)

Table 2: Moisture content of dry cassava (kokonte) flour from different Vendors

Sample code	Aym KF1	Aym KF2	Cm KF1	Cm KF2	Atm KF1	Atm KF2	Bm KF1	Bm KF2	CKF
Mean Moisture content/%	12.57 ± 0.34^{b}	13.03 ± 0.29^{b}	14.67 ± 0.23^{c}	16.90 ± 0.56^{d}	10.93 ± 0.27^{a}	11.03 ± 0.23^{a}	15.00 ± 0.06^{c}	13.00 ± 0.15^{b}	11.70± 0.17^{a}

The abbreviations indicate the markets, kokonte flour and vendors from whom the kokonte flours were bought.

Key: Aym - Ayigya market, Cm - Central market, Atm - Atonso market, Bm - Bantama market, CKF- Control for Kokonte Flour, KF- kokonte flour, 1 and 2 - Vendors 1 and 2 from the same market. Means that do not share the same letter (superscript) were significantly different (P<0.05) but those that share the same letter (superscript) do not differ significantly (P>0.05)

4.2. ENUMERATION OF MOULDS ISOLATED

Table 3 shows the mould count of the two flour samples bought from the various markets. Maize flour from Ayigya market recorded mould count of $3.03 \times 10^{5} \pm 0.96$ cfu/g and $1.67 \times 10^{3} \pm 0.30$ cfu/g for Vendor-1 and Vendor-2 respectively. In the case of the Central market, Vendor one had flour contaminants of $1.18 \times 10^{6} \pm 0.18$ cfu/g while flour from Vendor-2 recorded $3.40 \times 10^{3} \pm 0.17$ cfu/g as the fungal count. Samples from Bantama market did not record any mould count for both

Vendors but for Atonso market, Vendor-2 recorded 2.97 $\times 10^3 \pm 0.30$ cfu/g while Vendor-1 recorded no count. There was no count for the control maize flour sample. For the dry cassava (kokonte) flour, samples from Ayigya market recorded mould count of 1.08 $\times 10^4 \pm 0.25$ cfu/g and 1.90 $\times 10^5 \pm 0.25$ cfu/g for Vendor-1 and Vendor-2 respectively whereas that from Central market had 3.20 $\times 10^4 \pm 0.32$ cfu/g and 4.03 $\times 10^5 \pm 0.35$ cfu/g counts for Vendor-1 and Vendor-2 respectively. Atonso market recorded 1.77 $\times 10^4 \pm 0.18$ cfu/g as the mould count for Vendor-1 and 7.03 $\times 10^4 \pm 0.61$ cfu/g for Vendor-2. Counts in samples from Bantama market for both Vendor-1 and Vendor-2 were 1.43 $\times 10^4 \pm 0.18$ cfu/g and 1.70 $\times 10^3 \pm 0.15$ cfu/g respectively. The control sample recorded growth that was too few to count (TFTC).

Table 3: Enumerated Moulds on the samples

	MARKETS				
FLOUR SAMPLES	Aym	Cm	Atm	Bm	C
MF1	$3.03 \times 10^5 \pm 0.96^a$	$1.18 \times 10^6 \pm 0.18^b$	NIL[a]	NIL[a]	NIL[a]
MF2	$1.67 \times 10^3 \pm 0.30^a$	$3.40 \times 10^3 \pm 0.17^a$	$2.97 \times 10^3 \pm 0.30^a$	NIL[a]	
KF1	$1.08 \times 10^4 \pm 0.25^c$	$3.20 \times 10^4 \pm 0.32^c$	$1.77 \times 10^4 \pm 0.18^c$	$1.43 \times 10^4 \pm 0.18^c$	TFTC
KF2	$1.90 \times 10^5 \pm 0.25^d$	$4.03 \times 10^5 \pm 0.35^e$	$7.03 \times 10^4 \pm 0.61^c$	$1.70 \times 10^3 \pm 0.15^c$	

Key: C – control, TFTC- Too Few To Count, NIL – No mould count

4.3. IDENTIFIED MOULDS

Moulds belonging to five genera were isolated and identified from both the maize flour and the dry cassava (kokonte) flour. The different genera included *Cladosporium*, *Aspergillus*, *Mucor*, *Rhizopus* and *Penicillium* (Figure 1). Tables 4 and 5, show the different organisms isolated from the various flour samples from the different markets. Thirteen (13) different mould species were isolated from the maize flour while ten (10) were isolated from the "kokonte" flour samples. Five different species of *Aspergillus*, three of *Penicillium* species, two *Cladosporium* species, two *Mucor* species and one *Rhizopus* species were identified in maize flour samples bought from the various markets. For the dry cassava (kokonte) flour samples, four species of *Aspergillus* were identified, while two species each of *Penicillium* and *Mucor* and one species each of *Rhizopus* and *Cladosporium* were also identified.

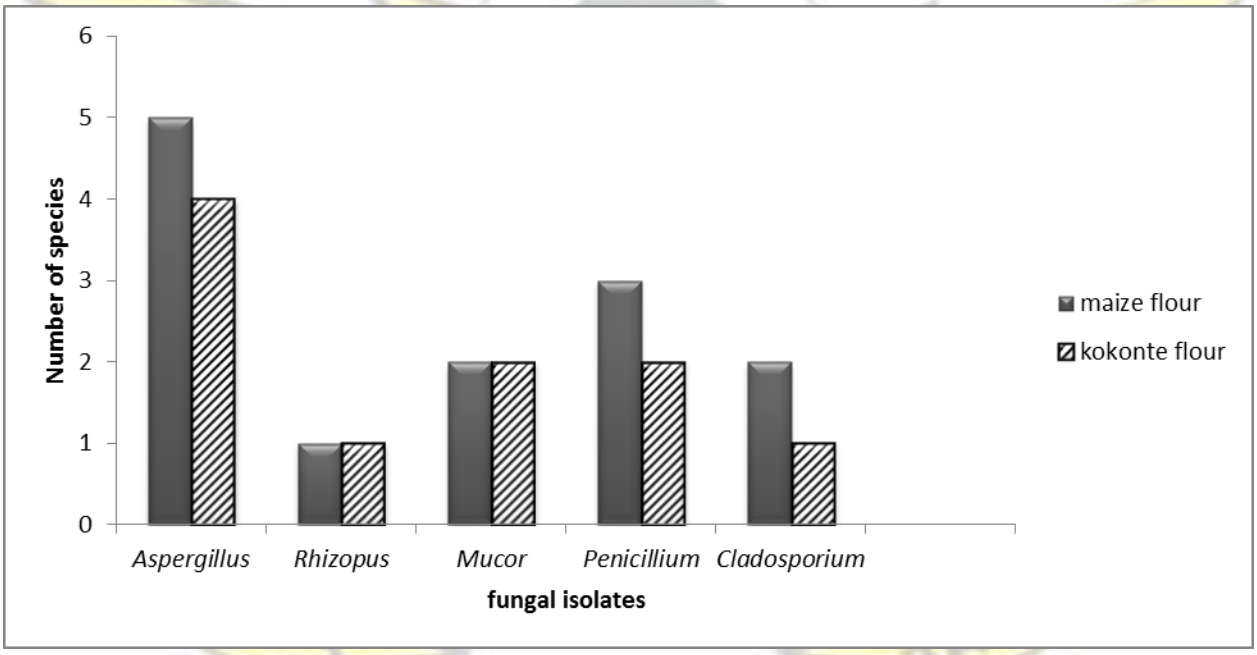

Figure 1: Types of moulds and levels identified in the samples

Table 4: Moulds isolated from maize flour samples from the different market

FLOUR SAMPLE	ORGANISM ISOLATED
Aym MF1	*Mucor racemosis, Cladosporium cladosporioides, Aspergillus flavus, Aspergillus fumigatus, Aspergillus wentii, Aspergillus ochraceus, Aspergillus versicolor, Cladosporium herbarum, Penicillium crustosum, Penicillium camemberti*
Aym MF2	*Aspergillus ochraceus, Aspergillus wentii, Aspergillus fumigatus, Cladosporium cladosporioides, Penicillium crustosum*
Cm MF1	*Rhizopus stolonifer, Penicillium crustosum, Mucor racemosis,*
Cm MF2	*Aspergillus flavus, Rhizopus stolonifer, Cladosporium cladosporioides, Aspergillus wentii,*
Atm MF1	No observable growth recorded
Atm MF2	*Mucor hiemalis, Rhizopus stolonifer, Penicillium crustosum, Penicillium viridicatum*
Bm MF1	No observable growth recorded
Bm MF2	No observable growth recorded
CMF	No observable growth recorded

Table 5: Moulds isolated from dry cassava (kokonte) flour samples from the different markets

FLOUR SAMPLE	ORGANISM ISOLATED
Aym KF1	*Aspergillus flavus, Aspergillus wentii*
Aym KF2	*Aspergillus flavus, Penicillium crustosum, Cladosporium cladosporioides, Aspergillus wentii*
Cm KF1	*Rhizopus stolonifer, Aspergillus flavus, Penicillium crustosum, Cladosporium cladosporioides*
Cm KF2	*Rhizopus stolonifer, Cladosporium cladosporioides, Aspergillus flavus, Aspergillus wentii, Mucor hiemalis, Mucor racemosis,*
Atm KF1	*Aspergillus flavus, Rhizopus stolonifer, Mucor hiemalis, Penicillium viridicatum*
Atm KF2	*Aspergillus flavus, Mucor racemosis, Mucor hiemalis, Rhizopus stolonifer*
Bm KF1	*Mucor hiemalis, Rhizopus stolonifer, Aspergillus flavus, Penicillium viridicatum, Aspergillus versicolor, Aspergillus fumigatus*
Bm KF2	*Aspergillus fumigatus, Mucor hiemalis, Mucor racemosis Rhizopus stolonifer*

CKF	*Cladosporium cladosporioides, Penicillium crustosum,*

4.4. PERCENTAGE DISTRIBUTION OF MOULD SPECIES ISOLATED

From the study carried out, *Aspergillus flavus* was found to be the most prevalent mould species in the dry cassava (kokonte) flour occurring in seven of nine samples representing 77.8%. *R. stolonifer* occurred in six of nine samples (66.7%), *M. hiemalis* occurred in five of nine samples (55.6%) and *C. cladosporioides* in three out of nine samples (44.4%). *A. wentii* and *M. racemosis* were present in 33.3% of the samples while *A. fumigatus, P. crustosum,* and *P. viridicatum* were found in 22.2% of the samples. *A. versicolor* was the least prevalent in the samples occurring in only one of the sample representing 11.1%.

For the maize flour samples, the most dominant species was *P. crustosum* occurring in 44.4% of the samples, followed by *A. wentii, C. cladosporioides* and *R. stolonifer* occurring in three of nine samples (33.3%). *Aspergillus flavus, A. fumigatus* and *M. racemosis* occurred in two of the samples representing 22.2%, while *A. versicolor, M. hiemalis, P. viridicatum, P. camemberti, C. herbarum* and *A. ochraceus* occurred in one of the samples representing 11.1% (Figure 2).

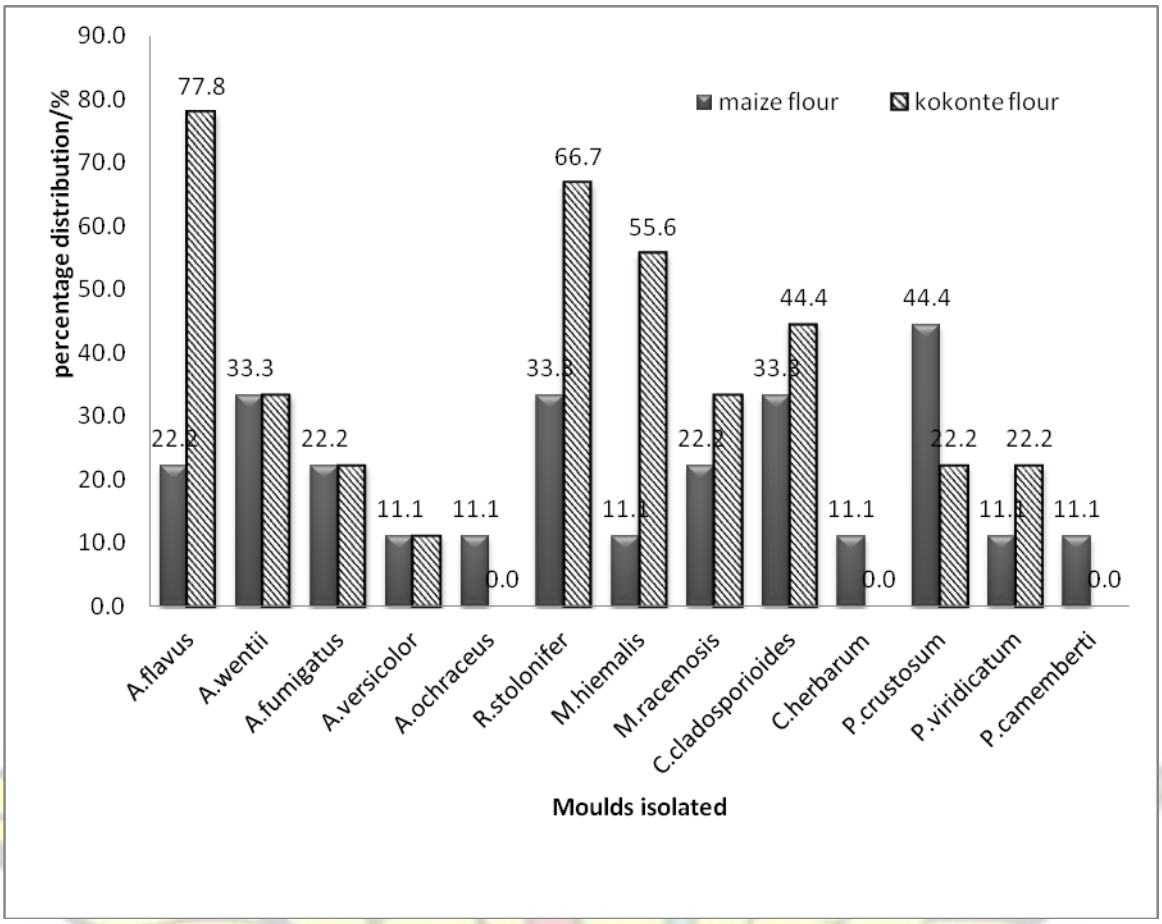

Figure 2: Percentage distribution of mould isolates in the flour samples

Culture Plates

Plate 3: Representative plate of pure culture of *Aspergillus wentii* from kokonte flour

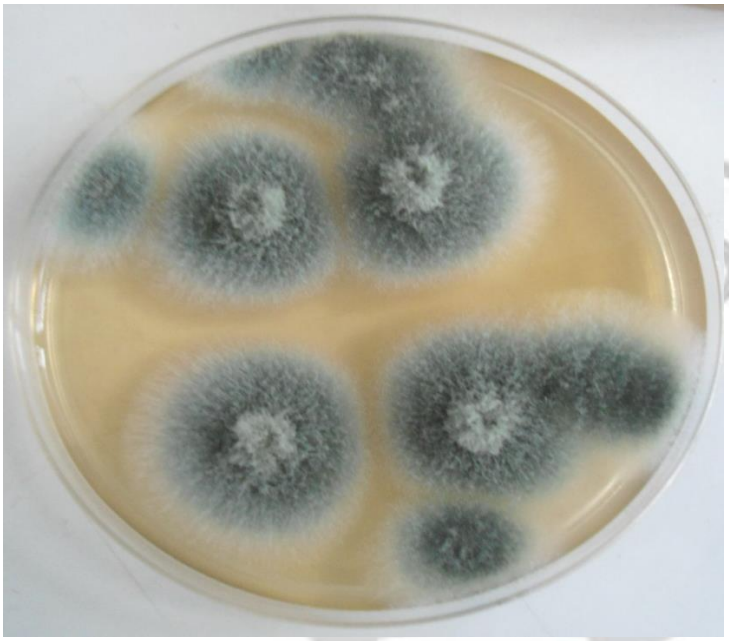

Plate 4: Representative plate of pure culture of *Aspergillus fumigatus* from maize flour

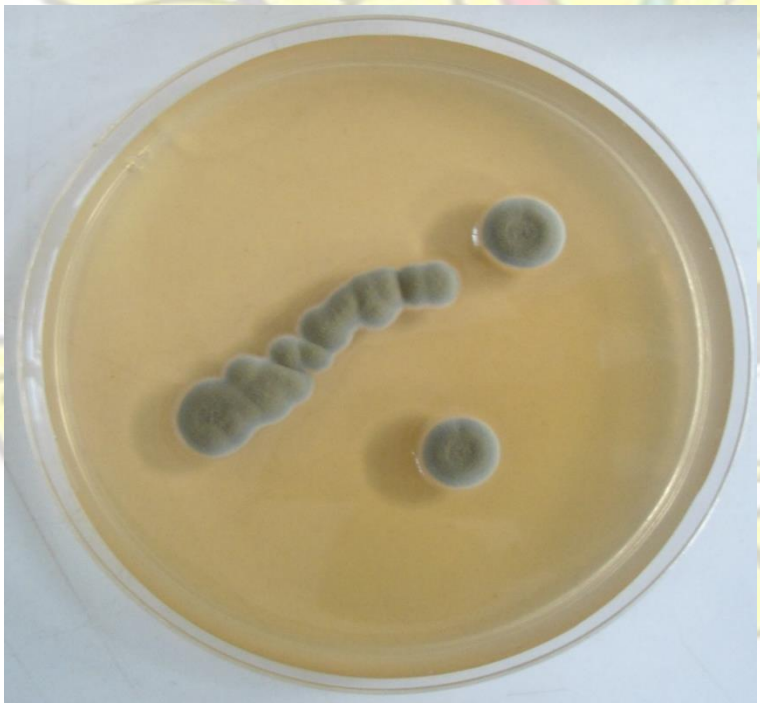

Plate 5: Representative plate of pure culture of *Cladosporium herbarum* from maize flour

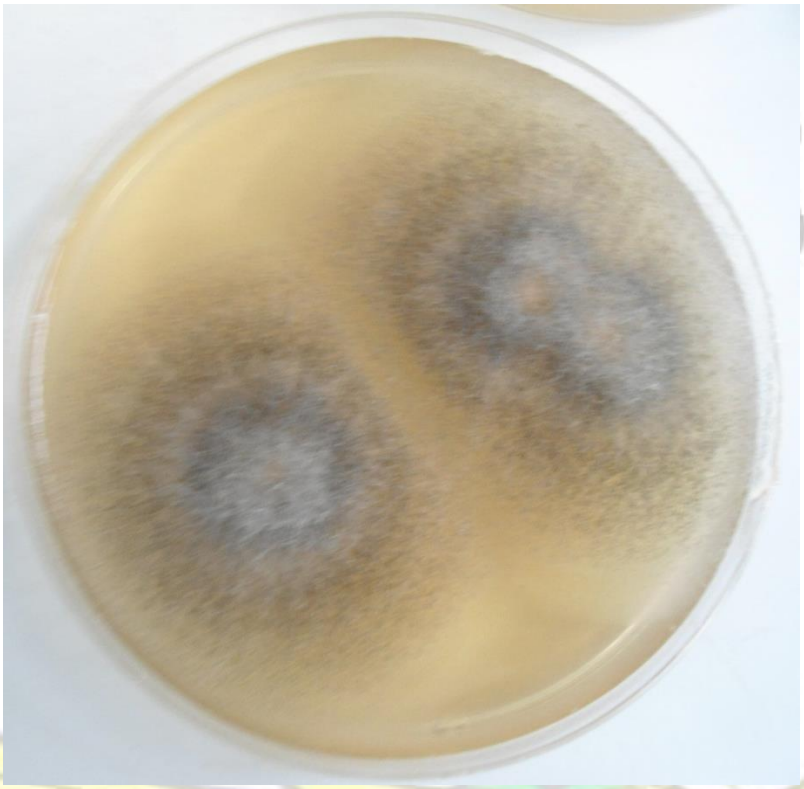

Plate 6: Representative plate of pure culture of *Mucor hiemalis* from maize flour

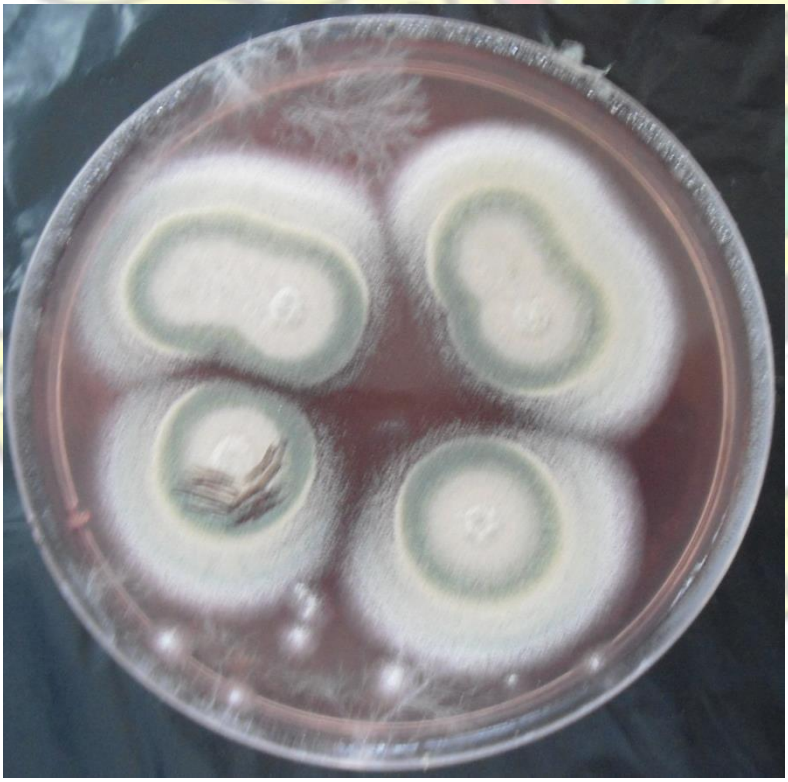

Plate 7: Representative plate of pure culture of *Aspergillus versicolor* from kokonte flour

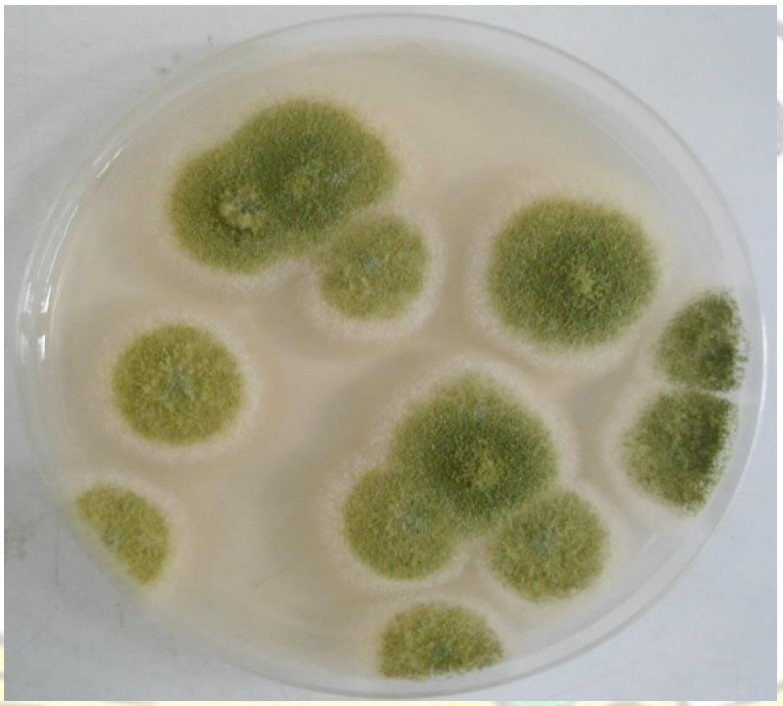

Plate 8: Representative plate of pure culture of *Aspergillus flavus* from kokonte flour

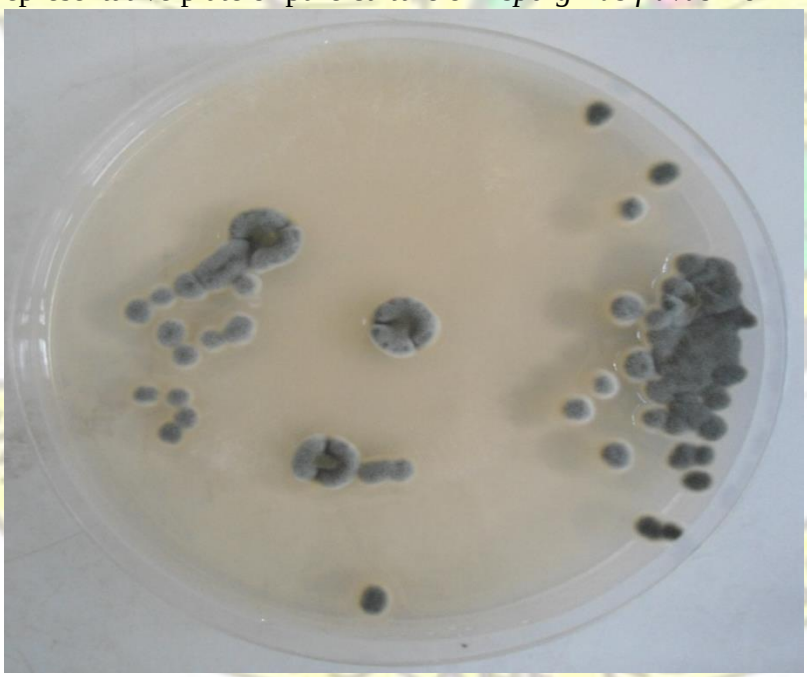

Plate 9: Representative plate of pure culture of *Cladosporium cladosporioides* from maize flour

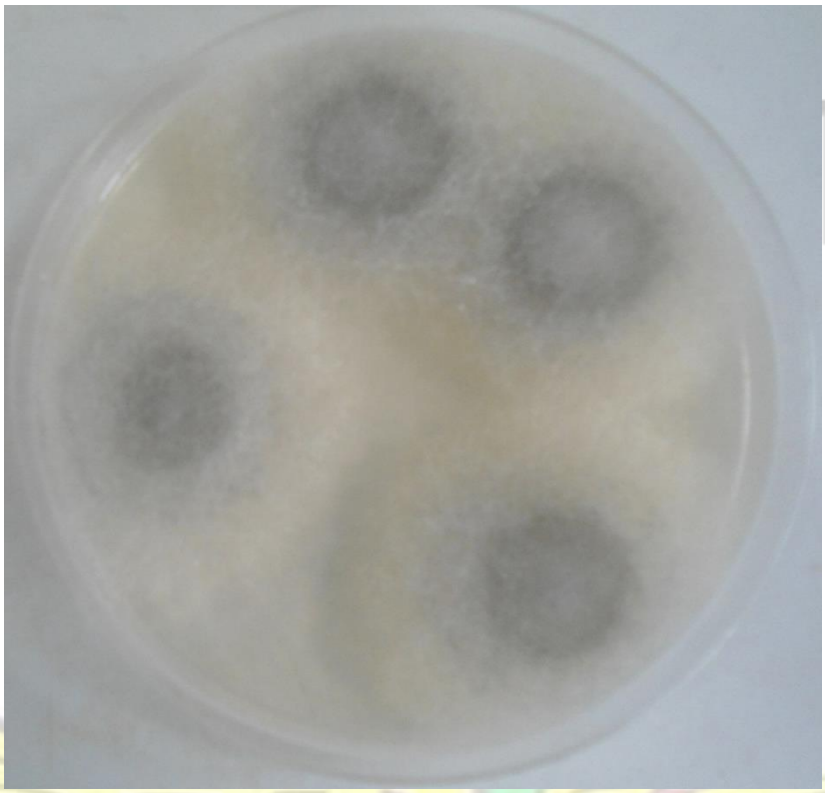

Plate 10: Representative plate of pure culture of *Mucor racemosus* from maize flour

Plate 11: Representative plate of pure culture of *Rhizopus stolonifer* from kokonte flour

4.5. COLIFORM COUNT

Most of the samples tested negative for coliforms except for maize flour sample bought from Vendor2 from Atonso market (Atm MF2), dry cassava (kokonte) flour bought from the central market Vendor-1(Cm KF1) and "kokonte flour" bought from Vendor-1(Bm KF1) (Table 6).

Table 6: Coliform test on samples

Sample	Test result	Sample	Test results
Aym MF1	Negative	Aym KF1	Negative
Aym MF2	Negative	Aym KF2	Negative
Cm MF1	Negative	Cm KF1	Positive
Cm MF2	Negative	Cm KF2	Negative
Atm MF1	Negative	Atm KF1	Negative
Atm MF2	Positive	Atm KF2	Negative
Bm MF1	Negative	Bm KF1	Positive
Bm MF2	Negative	Bm KF2	Negative
CMF	Negative	CKF	Negative

4.6. TOTAL PLATE COUNT

Table 7 shows the total plate count of organisms isolated from all the flour samples under study. From the results obtained, maize flour from Atonso market Vendor-2 (Atm MF2) recorded the highest count ($9.1 \times 10^6 \pm 0.25$ cfu/g) among the maize flour samples while that from Bantama market Vendor-1 (Bm MF1) recorded the least (no growth). For the dry cassava (kokonte) flour samples, sample from the Central market Vendor-2 (Cm KF2) recorded the highest count ($4.64 \times 10^6 \pm 3.18$ cfu/g), whereas the control dry cassava (kokonte) flour sample (CKF) recorded the lowest count of $5.63 \times 10^3 \pm 0.45$ cfu/g.

Table 7: Total plate count (cfu/g) of organisms isolated from both flour samples

	MARKET				
FLOUR SAMPLE	Aym	Cm	Atm	Bm	C
MF1	$5.5 \times 10^4 \pm 0.62^a$	$4.02 \times 10^5 \pm 3.05_{a, b}$	$3.4 \times 10^3 \pm 0.62_a$	NIL[a]	$4.37 \times 10^3 \pm 0.83_a$
MF2	$6.4 \times 10^5 \pm 0.30_{a, b}$	$8.7 \times 10^4 \pm 0.96^a$	$9.1 \times 10^6 \pm 0.25^c$	$3.42 \times 10^6 \pm 2.52_b$	
KF1	$3.3 \times 10^6 \pm 2.41^e$	$6.4 \times 10^5 \pm 0.78^e$	$4.43 \times 10^6 \pm 3.03^e$	$4.33 \times 10^5 \pm 3.35^e$	$5.63 \times 10^3 \pm 0.45^e$
KF2	$4.6 \times 10^5 \pm 0.62^e$	$4.64 \times 10^6 \pm 3.18^e$	$3.76 \times 10^6 \pm 2.69^e$	$7.8 \times 10^3 \pm 0.30^e$	

4.7. DATA FROM QUESTIONNAIRE ADMINISTERED

The questionnaire captured information on the period the samples had been on the shelves, the method of drying, how samples were packaged, the hygienic condition surrounding the samples, and the educational background of the vendors. The data revealed that, the duration of the samples on the shelves ranged from one to three days. With the method of drying, most vendors dried the samples on rubber spread on the ground, and in terms of packaging, most vendors had their samples not enclosed (eight of sixteen), five had samples enclosed in rubber while three had their samples partially enclosed. Twelve vendors were selling under good hygienic condition while four sold under poor hygienic condition. The data also showed that eight of the vendors had no formal education, six acquired education to the junior level while only two received secondary education (Table 8).

Table 8: Data from questionnaires

SAMPLES	SHELF LIFE/ DAYS	METHOD OF DRYING	PACKAGE	HYGIENE LEVEL	EDUCATION LEVEL	GENDER
Aym MF1	3	spread on a rubber laid on the ground	not enclosed	Good	NFE	Female
Aym MF2	2	spread on a rubber laid on the ground	not enclosed	Good	NFE	Female
Cm MF1	3	spread on a rubber laid on the ground	not enclosed	Good	JL	Female
Cm MF2	1	spread on a rubber laid on the ground	not enclosed	Poor	NFE	Female
Atm MF1	1	spread on a rubber laid on the ground	enclosed in rubber	Good	JL	Female

Atm MF2	2	dried on a bed above the ground	not enclosed	Poor	NFE	Female
Bm MF1	1	spread on a rubber laid on the ground	enclosed in rubber	Good	JL	Female
Bm MF2	1	spread on a rubber laid on the ground	partially enclosed	Good	SL	Female
CMF	1	solar drier				
Aym KF1	2	spread on a rubber laid on the ground	not enclosed	Good	NFE	Female
Aym KF2	3	spread on a rubber laid on the ground	not enclosed	Good	NFE	Female
Cm KF1	3	spread on a rubber laid on the ground	enclosed in rubber	Good	JL	Female
Cm KF2	3	spread on a rubber laid on the ground	not enclosed	Poor	NFE	Female
Atm KF1	2	spread on a rubber laid on the ground	partially enclosed	Good	JL	Female
Atm KF2	1	spread on a rubber laid on the ground	enclosed in rubber	Poor	NFE	Female
Bm KF1	2	spread on a rubber laid on the ground	enclosed in rubber	Good	JL	Female
Bm KF2	2	spread on a rubber laid on the ground	partially enclosed	Good	SL	Female
CKF	1	solar drier				

Key: NFE-No Formal Education, SL- Senior Level, JL- Junior Level

CHAPTER FIVE

5.0. DISCUSSION

Results obtained from the experiment indicated that processed indigenous maize flour and dry cassava (kokonte) flour sold on the selected markets were contaminated with different kinds of microorganisms. This was confirmed by the different array of organisms isolated from the various samples. For the dry cassava (kokonte) flour, all the samples were contaminated with moulds, representing 100% moulds contamination, but the levels of contamination varied among the samples. The level of mould contamination among the samples exceeded the acceptable moulds level in food (10^3 cfu/g) as reported by African Organization for Standardization, (2012). This is an indication that the dry cassava (kokonte) flour sold on the various markets may be unwholesome and, therefore, pose health risk to consumers. In comparing the results for mould count in the various "kokonte" flour, with results obtained by Lu *et al*., (1988), all the samples recorded count higher than 6.5×10^3 cfu/g as they reported in their studies except for the sample from Vendor-2 in Bantama market (Bm KF2) which recorded $1.70 \times 10^3 \pm 0.15$ cfu/g. The control sample also recorded count lower than reported. Statistical analysis showed a significant difference between the counts at $p<0.05$. The high incidence of contamination in these samples may be attributed to factors such as the high moisture content of the flour samples, the length/period the samples have been on market, the processing method, the hygienic practices employed in processing, and the condition under which the food commodity was sold on the market.

The moisture content obtained for the various "kokonte" flour samples (ranging from 10.93% to 16.90%) is in agreement with the study result (10.0% to 16.9%) as reported by Lokko, (1978).

Several researches have been conducted to establish an acceptable moisture content of "kokonte" flour. Report by Lokko, (1978) showed that for a kokonte flour to remain stable, a moisture content of 12% is required. Apart from samples bought from Atonso market (Atm KF1 and Atm KF2) and the control (CKF) which recorded value lower than 12%, the remaining samples had moisture contents higher than 12%. This suggests that the high moisture content may be a contributing factor to the source of contamination since studies have shown that microorganisms require moisture for their growth (FDA, 2015). Correlation analysis revealed a positive correlation between moisture content and mould count of dry cassava (kokonte) flour. Though high moisture content in foods is known to be a strong influence for growth of microorganisms, the results for the moisture content of Atm KF1, Atm KF2 and CKF from this study suggest that other factors apart from the high moisture content may account for the contamination in the samples.

Results from coliform test, which is an indicator of personal hygiene level of flour sellers (Victor *et al*., 2013), recorded six samples to be negative representing 75% of the samples. Inference from this test suggests that the high level of contamination may not necessarily be as a result of poor hygiene practices by flour sellers. This is in agreement with data collected from questionnaire as about 75% of sellers were selling under good hygienic conditions.

Comparing the mould count of the various "kokonte" samples to the control sample (CKF), it can be suggested that the main source of the contamination may be due to some processing steps as suggested by Aworh, (2008). Comparing the processing steps to that of the control, it would be realized that, safety precautions may not have been followed in the line of production. For instance, data from the questionnaire showed that the raw material (cassava) used was not washed before peeling and this cuts across for all the sellers, meanwhile washing raw material before peeling is a way of reducing the microbial load on raw material after harvesting. In the case of the control

sample, it was washed thoroughly before peeling. The cassava samples after peeling were also washed once by all the processors which may not be efficient in reducing the microbial load on the raw material. The control sample, on the other hand, was washed three times with clean water after it was peeled. Furthermore, for all the samples, drying of cassava chips was done on a rubber mat laid on the ground as compared with the control sample which was dried using a solar drier. Studies have shown that the source of mould contamination may be from the soil or air since they are ubiquitous in nature (Victor *et al.*, 2013), and this therefore suggest that open sun drying may be another source of the contamination. The mode of packaging can also be another source of contamination to the samples. For instance, Aym KF2 and Cm KF2 which recorded the highest moulds count of $1.90 \times 10^5 \pm 0.25$ cfu/g and $4.03 \times 10^5 \pm 0.35$ cfu/g, respectively were not enclosed in any package; however, the Bm KF2 which was partially enclosed recorded the least count of $1.70 \times 10^3 \pm 0.15$ cfu/g.

In this study five different types of moulds belonging to the genera; *Mucor, Rhizopus, Aspergillus, Penicillium* and *Cladosporium* were isolated from the various "kokonte" flour samples and this is contrary to a study by Lu *et al.*, (1988) who isolated only two different genera; *Aspergillus* and *Penicillium* from their samples. The most predominant organisms isolated from the dry cassava (kokonte) flour were *Aspergillus flavus, Rhizopus stolonifer* and *Mucor hiemalis* occurring in 77.8%, 66.7% and 55.6% of the samples respectively.

Results from the enumeration of moulds in the maize flour samples revealed varying degree of contamination among the samples ranging from no observable mould count as recorded by Atm MF1, Bm MF1, Bm MF2 and CMF to high count of $1.18 \times 10^6 \pm 0.18$ cfu/g as recorded by Cm MF1. Samples from Aym MF2, Cm MF2 and Atm MF2 showed counts that were a little higher than the tolerable level of 10^3 cfu/g, recording $1.67 \times 10^3 \pm 0.30$ cfu/g, $3.40 \times 10^3 \pm 0.30$ cfu/g and

$2.97 \times 10^3 \pm 0.30$ cfu/g respectively. Extremely low counts of mould in the samples as recorded by Atm MF1, Bm MF1, Bm MF2 and CMF is in agreement with results obtained by Adu-Gyamfi and Appiah, (2012) obtaining mould count of 5.0×10^1 cfu/g. The low count of moulds reported may be due to the dehulling of grains before processing into flour as reported by Victor *et al.*, (2013). It is thought that the microorganisms are usually found on the outer coat of the grains and hence dehulling is a means of reducing contamination. The extent of contamination in samples for Aym MF1 and Cm MF1, however, suggests that the dehulling process may not be a guarantee that samples are absolutely free from contaminants. The high moisture content recorded by these samples suggested the existence of favourable condition for growth of microorganisms. The open air method of drying samples may also be another source of contamination as it may expose the samples to spores and other microbes in the atmosphere. Observing the trend of days the sample had been on the market, revealed that for all the samples that had low mould count had been on the shelves for only one day as compared to three days recorded by the heavily contaminated samples. Correlation analysis shows a strong correlation (P= 0.725) between mould count and period the sample has been on the shelf. Package of samples may also contribute to the contamination of the sample. Data from the questionnaire showed that maize flour samples that were contaminated were actually not enclosed in any package hence exposing sample to fungal spores in the air. Coliform test, which is an indicator of personal hygiene reported negative for seven of the eight samples, thus, it may be suggested that the source of contamination did not directly emanate from the Vendors but rather activities involved in the processing chain (Aworh, 2008).

Thirteen different mould species belonging to five genera were isolated from the maize flour samples including *Aspergillus, Penicillium, Cladosporium, Mucor,* and *Rhizopus*. Five different species of *Aspergillus* were isolated, three *Penicillium* species, two of *Cladosporium* and *Mucor*,

and one *Rhizopus* species. The most predominant species were *Penicillium crustosum, Rhizopus stolonifer, Cladosporium cladosporioides* and *Aspergillus wentii.*

The prevalence of these organisms in both samples (maize flour and kokonte flour) above the acceptable threshold means consumers may be at risk of biological hazards. *Aspergillus flavus* was the most dominant species in the cassava (kokonte) flour occurring in 77.8% of the samples. Studies have shown that moulds such as *Aspergillus flavus* produce mycotoxins (aflatoxins) which are known to be teratogenic, mutagenic, hepatotoxic, genotoxic and hepato carcinogenic depending on how long an individual gets exposed to the toxin (Fung and Clark, 2004).

Aflatoxin contamination in maize was attributed to the cause of aflatoxic hepatitis outbreak in Kenya and India (Matumba *et al*., 2009). *A. flavus* is a saprophytic fungi which inhabits in the soil and cause infection to both field crops, pre-harvested and post- harvested crops (Amaike and Keller, 2011). The presence of these organisms in both maize flour and "kokonte" flour has been reported by Daniel *et al.,* (2011) and Chiona *et al.*, (2014) respectively.

Aspergillus ochraceus is another species of *Aspergillus* whose ecological root is the soil though they may also be found in other environmental niches (Ghibaudo and Peano, 2010). Studies show that this fungus is known to cause infections to several crops including maize *(*Wilson *et al.,* 2002). It is known to produce of one of the most abundant food-contaminating mycotoxins known as ochratoxin A which has been found to have strong carcinogenic effect on the kidney and liver of humans (Li and Ji, 2003). The occurrence of *A. ochraceus* in maize flour compares with a study carried out by Alborch *et al*., (2012).

Aspergillus fumigatus like other *Aspergillus* species is a saprophyte found in the soil, decaying organic materials and also widely spread in nature. The prevalence of this organism in the various flour samples may be due to the ubiquitous nature of their spores as mentioned by O"Gorman *et*

al., (2009). *A. fumigatus* has been described as an opportunistic human pathogen which causes potentially lethal invasive infection in immunocompromised people associated with severe asthma and sinusitis (O"Gorman *et al*., 2009).

Penicillium spp. are among the common moulds which occupy the mycoflora of maize and cassava flour due to their presence in the soil and ubiquitous nature (Alborch *et al.,* 2012). Studies have shown that the ability of these organisms to survive in food items with low moisture content is due to their propensity to thrive in low humidity and also colonize rapidly (Pitt, *et al.*, 2000). Isolation of *Penicillium* species in maize flour agrees with studies carried out by Victor *et al*., (2013). *Penicillium crustosum* isolated from both flour samples is known to produce neurotoxins called penitrem A which has serious toxic effect on the nervous system (MoldesAnaya, 2011). *Penicillium viridicatum* is also known to produce ochratoxin A or citrinin in grains (Pitt, 1987), toxins that are potentially carcinogenic to humans and also proposed to induce reduction in antioxidant defenses (Cavin *et al*., 2007). *Penicillium camemberti* is an important fungus associated with the production of cheese but its presence in the various flour samples may be attributed to the ubiquitous nature of moulds in general. *P. camemberti* is also known to produce cyclopiazonic acid, a toxin which is toxic at high concentrations (Sosa *et al*., 2002).

Rhizopus stolonifer also known as black mould is a common mould that grows on bread; however, these organisms have their spores floating in the air hence their presence in the flour may be due to exposure of samples to open air. These organisms are fast growing, producing black spores and contaminating culture plate hence growth on plate was closely monitored to avoid overgrowth. The prevalence of these organisms in maize flour has also been confirmed by Rahmawati *et al.*, (2013). Report from Espinel-Ingroff *et al.*, (1987), showed that there is no strong evidence to support the pathogenicity of *Rhizopus stolonifer* in humans since they do not grow at 37 ^{o}C.

Cladosporium are moulds that are found on both living and dead plant materials (CDC, 2015). They are known to be among the most common indoor and outdoor moulds hence a contributing factor to their presence in the flour samples. The presence of *Cladosporium cladosporioides* in the maize flour samples in this study is in agreement with report by Allotey *et al.*, (2001). *Cladosporium* species are not known to produce mycotoxins and are rarely pathogenic to humans, however, studies have shown that spores produced by these organisms may produce allergic reaction in asthmatic patients and people with respiratory problems (Hasnain *et al*., 2004). Two different species of this organism were isolated from the maize flour sample (*C. cladosporioides* and *C. herbarum*) while only one species was isolated from the cassava flour samples (*C. cladosporioides*). The presence of C*ladosporium* spp. in the dry cassava (kokonte) flour samples has also been discussed by Wareing *et al.*,(2008).

Mucor species are among the diverse group of fungi found in the soil, growing on decay foods, manure, plants among other. These organisms are not infectious to human as they are unable to grow at temperature close to 37 ^{o}C (MBL, 2015). Two Mucor species *(M. racemosis* and *M. hiemalis*) were isolated from both "kokonte" and maize flour samples. The occurrence of these organisms in the samples may be due to pre-contamination of the raw material since they are found growing in the soil or may be due to exposure of samples to open air during preparation of the flour. The presence of *Mucor* spp. in cassava and maize flours has been reported by Wareing *et al*., (2008).

Results from the total plate count is an important indication of the hygienic conditions surrounding the food and also shows the effectiveness and efficiency of the food chain process as well as the shelflife of the food (Victor *et al*., 2013). In this study, the total plate count from the "kokonte" samples were higher (Table 6) than that reported by Lu *et al*., (1988) who recorded 16

$\times 10^3$ cfu/g. Only samples from Vendor-2 in Bantama market (Bm KF2) and the control "kokonte" flour (CKF) recording $7.8 \times 10^3 \pm 0.30$ cfu/g and $5.63 \times 10^3 \pm 0.45$ cfu/g, respectively were a little lower than the results obtained by Lu *et al*., (1988). For most of the samples, the level of contamination was found to be higher than the recommended level (10^5 cfu/g) and this is an indication of poor sanitation or problems resulting from the process control or handling of the raw material (Victor *et al.*, 2013).

For the maize flour samples, except for samples from Bantama market Vendor-1 (Bm MF1) which did not record any growth for total plate count, the remaining samples recorded counts which exceeded the tolerable level. Results from the total plate count of all the 16 samples (except maize flour from Bantama market Vendor-1) indicate that favourable conditions exist within the flour to support the growth of various organisms. The high incidence of contamination in the maize flour may be attributed to the high moisture contents observed in all the samples as presented in Table 1. The high total plate count obtained from this study is in agreement with report from Victor *et al*., (2013) in their study on microbial and physicochemical characterization of maize flour.

Data from the questionnaire showed that, all (16) the Vendors were females, eight never had any formal education, six were junior level leavers and two had attained secondary education. This information showed that the vendors may have little or no knowledge on microbial contamination of food hence, little awareness on the safety of foods. It was also revealed that, all the Vendors prepared the maize flours themselves, but with the cassava flour, most of the Vendors (five out of eight) bought the dry cassava chips from different towns and process them into the flour for sale. Due to this, they had little knowledge as to how the samples were treated before they bought them but the information captured on how the samples were prepared was based on their

knowledge in the production process. All the samples were milled using a commercial milling machine and this could also be another source of contamination in the samples.

CHAPTER SIX

6.0. CONCLUSIONS AND RECOMMENDATIONS

6.1. CONCLUSIONS

This study revealed that most of the processed indigenous flours sold on the market had high moisture content which provided favourable conditions for the growth of microorganisms. For the moulds count, except for samples CKF, AtmMF1, CMF, BmMF1 and BmMF2 which recorded counts below the acceptable level of 10^3 cfu/g, the remaining samples (both maize flour and cassava flour) recorded counts above the tolerable level. In all thirteen different mould species belonging to five genera were isolated from both flour samples. Ten of which were identified in the dry cassava flour while all thirteen were isolated from the maize flour samples.

The total plate count recorded values between no counts to values above the tolerable level of 10^5 cfu/g. 50 % of maize flour samples and 87.5 % of dry cassava (kokonte) flour samples bought from the various markets showed counts above the tolerable level but the control samples recorded counts below the acceptable level. The coliform test reported negative for seven out of eight (7/8) maize flour sample while six of eight (6/8) was recorded for the kokonte flour sample. Both control samples also recorded negative.

The information obtained from this studies shows that processed indigenous flours get contaminated by various organisms above the acceptable threshold which may to some extent affect the health of consumers.

6.2. RECOMMENDATIONS

It is recommended that studies should be carried out to determine the critical points along the production chain so that interventions would be more effective. It is also recommended that mycotoxin levels should be assessed to ensure that indigenous flours are safe for consumption.

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APPENDICES

APPENDIX I: MEDIA PREPARATION

A: Potato Dextrose Agar (PDA)

PDA is general purpose media for the isolation of fungi. The medium was prepared by dissolving 39 grams of the dehydrated powder in one litre of distilled water. It was heated over water bath to completely dissolve the agar and then sterilized by autoclaving at 121^{o}C for 15 minutes. After autoclaving it was allowed to cool and 100mg of chloramphenicol powder and 100mg of oxytetracycline was added to prevent growth of bacteria. The medium was mixed well, poured into petri dishes and allowed to solidify.

B: Oxytetracycline – Glucose Yeast Extract (OGYE) agar

OGY is a selective media for the isolation and enumeration of yeast and moulds. The medium was prepared by dissolving 37 grams of the dehydrated powder in one litre of distilled water. It was heated over water bath to completely dissolve the agar and then sterilized by autoclaving at 121^{o}C for 15 minutes. After autoclaving it was allowed to cool and 100mg of chloramphenicol powder was added to prevent growth of bacteria. The medium was mixed well, poured into petri dishes and allowed to solidify.

C: Dichloran Rose Bengal Chlortetracycline (DRBC) agar

DRBC is a selective media for the isolation and enumeration of yeast and moulds. The medium was prepared by dissolving 31.5 grams of the dehydrated powder in one litre of distilled water. It was heated over water bath to completely homogenize the agar and then sterilized by autoclaving at 121^{o}C for 15 minutes. After autoclaving it was allowed to cool and 100mg of chloramphenicol powder and was added to prevent growth of bacteria. The medium was mixed well, poured into petri dishes and allowed to solidify.

D: Plate Count Agar (PCA)

PCA is a general purpose agar for the enumeration of bacteria. The medium was prepared by dissolving 17.5 grams of the dehydrated powder in one litre of distilled water. It was heated over water bath to completely dissolve the agar and then sterilized by autoclaving at 121^{o}C for 15 minutes. After autoclaving it was allowed to cool to about 50 ^{o}C, poured into sterile petri dishes and allowed to solidify.

E: MacConkey Broth

MacConkey broth is a selective and differential medium for the growth of coliforms and gram negative bacteria. The medium was prepared by dissolving 10 grams of the dehydrated powder in 250 mL of distilled water. 9 mL of the broth was transferred into test tubes and corked.

Sterilized was done by autoclaving at 121^{o}C for 15 minutes.

APPENDIX

II: Preparation of control samples

A: Kokonte flour

Fresh cassava was bought from the Ayigya market, washed thoroughly and peeled. The peeled cassava was washed three times with clean water and cut into chips for drying. The chips were dried in a solar drier for 72 hours and crushed with clean mortar and pestle to reduce the size of the chips. The crushed sample was milled into powder using a noncommercial milling machine, transferred into sterile bag and sent to the laboratory for analysis.

B: Maize flour

Dried maize grains were dehulled followed by soaking in water for three days to be fermented. The soaked grains were washed three times with clean water and milled. The milled samples were then dried in a solar drier for two days and milled again into fine powder. The sample was transferred into sterile bag and sent to the laboratory for analysis.

APPENDIX

III: Samples for laboratory work

A: Maize flour sample without enclosure

B: Partially enclosed kokonte flour

C: Drying cassava chips in solar drier

D: Open sun drying of maize flour

APPENDIX

V: Statistical Analysis

Table 9: ANOVA for moisture content in maize flour from the different Vendors

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	115.340	8	14.417	124.767	.000
Within Groups	2.080	18	.116		
Total	117.420	26			

Table 10: ANOVA for moisture content in kokonte flour from the different Vendors

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	94.896	8	11.862	47.519	.000
Within Groups	4.493	18	.250		
Total	99.390	26			

Table 11: ANOVA for moulds count between the maize flour samples

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6.511E12	8	8.139E11	15.164	.000
Within Groups	1.288E12	24	5.367E10		
Total	7.799E12	32			

APPENDIX

Table 12: ANOVA for mould count in the maize flour samples between the markets

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3.553E12	4	8.883E11	5.858	.001
Within Groups	4.246E12	28	1.516E11		
Total	7.799E12	32			

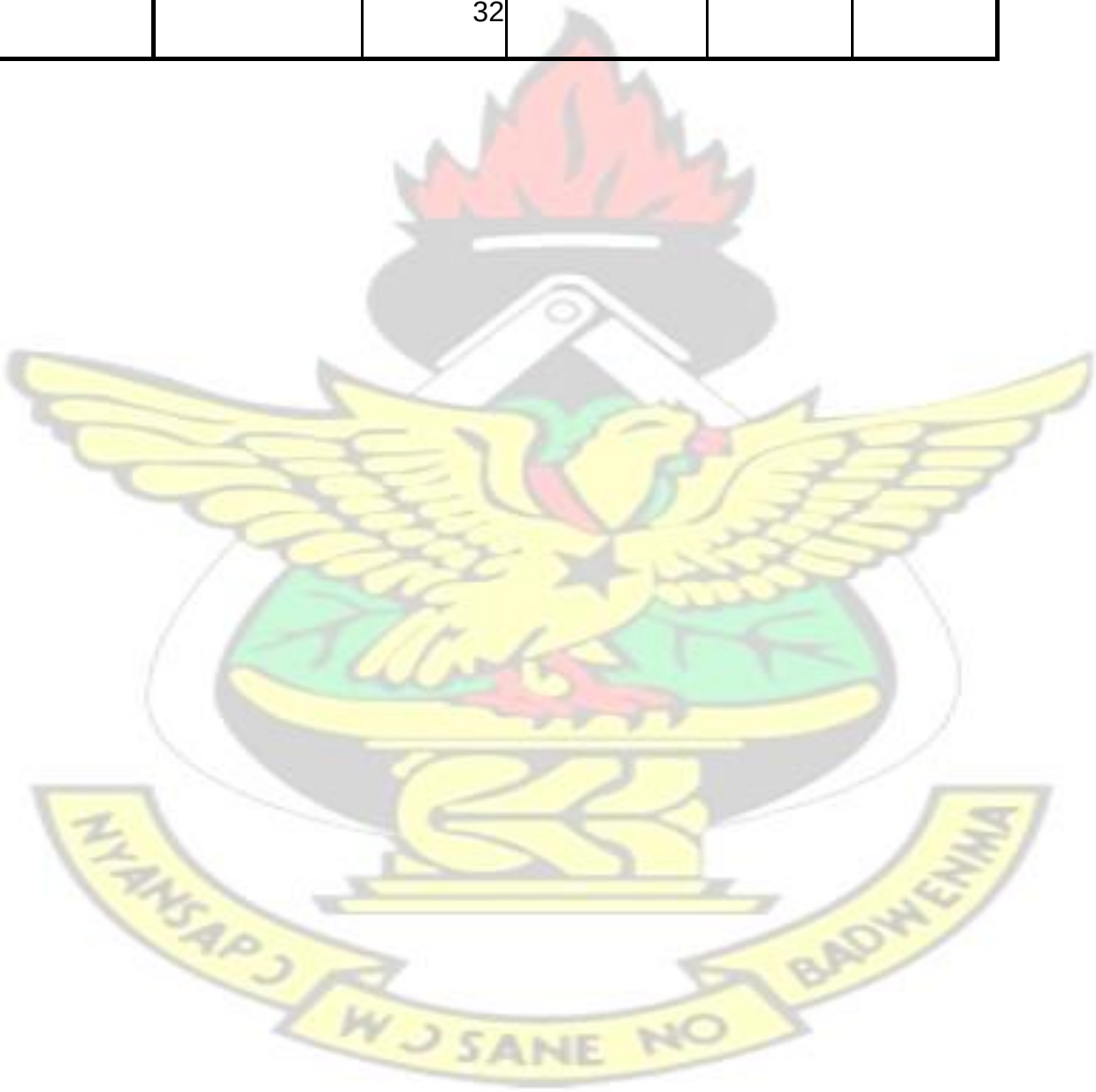

Table 13: ANOVA for moulds count between the kokonte flour samples

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4.653E11	7	6.647E10	53.250	.000
Within Groups	2.746E10	22	1.248E9		
Total	4.927E11	29			

Table 14: ANOVA for moulds count in the kokonte flour samples between the markets

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.554E11	3	5.180E10	3.993	.018
Within Groups	3.373E11	26	1.297E10		
Total	4.927E11	29			

Table 15: ANOVA for total plate count between the maize flour samples

	Sum of Squares	**Df**	**Mean Square**	**F**	**Sig.**
Between Groups	2.383E14	8	2.979E13	22.049	.000
Within Groups	3.243E13	24	1.351E12		
Total	2.708E14	32			

Table 16: ANOVA for total plate count in the maize flour samples between the markets

	Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Between Groups	8.917E13	4	2.229E13	3.437	.021
Within Groups	1.816E14	28	6.486E12		
Total	2.708E14	32			

Table 17: ANOVA for total plate count between the kokonte flour samples

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.500E14	8	1.875E13	3.858	.003
Within Groups	1.604E14	33	4.860E12		
Total	3.104E14	41			

Table 18: ANOVA for total plate count in the kokonte flour samples between the markets

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.004E14	4	2.509E13	4.421	.005
Within Groups	2.100E14	37	5.676E12		
Total	3.104E14	41			

APPENDIX V: Questionnaire

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

COLLEGE OF SCIENCE

DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY

QUESTIONNAIRE ON EVALUATION OF FILAMENTOUS FUNGI AND BACILLUS SPP. ON SELECTED PROCESSED INDIGENOUS FLOUR SOLD IN THE KUMASI METROPOLIS

I am a student of KNUST conducting a research evaluation of filamentous fungi on selected processed indigenous flour sold in the Kumasi Metropolis. Information received will be treated confidentially and used for academic purpose only.

(FOR FLOUR SELLERS)

1. Name of market ……
2. Name of sample: ……
3. Gender
   a. Male [ ] b. Female [ ]
4. Age range
   a. 15- 25 years [ ] b. 25-35 years [ ] c. 35-45 years [ ] d. 45 years and above [ ]
5. 2. Is that your only profession?
   a. Yes [ ] b) No [ ]
6. 3. Level of Education
   a. Primary level [ ] b. Junior level [ ] c. SHS level [ ] d. Tertiary level [ ]
7. Did you make the flour yourself?
   a. Yes [ ] b. No [ ]
8. If no, where did you buy it from? …….

Shelf life of the flour

9. When was the flour made
   a. One day [ ] b. two days [ ] c. three days [ ] d. four to six days [ ] e. no idea [ ]
10. How long has the flour been on your shelf
    a. One day [ ] b. two days [ ] c. three days [ ] d. four to six days [ ] e. beyond one week [ ]

How the flour was made

11. Was sample washed before peeling
    a. Yes [ ] b. No [ ]

12. Was sample washed before drying
    a. Yes [ ] b. No [ ]

13. If yes how many times was washing done before drying
    a. Once [ ] b. twice [ ] c. thrice [ ]

14. How was drying done
    a. In an oven [ ] b. by open sun drying [ ]
15. If in an oven at what temperature and how long? .....

16. If by open sun drying, where was drying done?
    a. On the bare ground [ ] b. on cemented floor [ ] c. spread over mat on the floor [ ]
    d. on a raised bed above the ground [ ]

17. How long was drying done
    a. 1 day [ ] b. 2 days [ ] c. 3-5 days [ ] d. above 5 days [ ]

18. Was the sample blanched before drying
    a. Yes [ ] b. No [ ]

19. If yes how long was it done
    a. 2-3 mins [ ] b. 4- 5 mins [ ] c. 6-7 mins [ ] d. 8-10mins [ ] e. above 10 mins

20. How was milling done
    a. Using personal mill [ ] b. using commercial mill [ ]

21. Was the milling machine cleaned before using to mill your sample?
    a. Yes [ ] b. No [ ]

Researcher's comment

22. How has the sample been packaged
    a. Enclosed in a rubber [ ] b. partially enclosed in a rubber [ ] c. not enclosed [ ]

23. How would you rank the hygienic conditions surrounding the sample
    a. Very good [ ] b. good [ ] c. poor [ ] d. very poor [ ]