



## Fungi Species Associated with Bread Spoilage in Birnin Kebbi Town, Kebbi State, Nigeria

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### ABSTRACT

Fungi associated with the spoilage of bread in Birnin Kebbi were investigated and identified. Three different bread samples were collected in Birnin Kebbi metropolis namely; Kampala, Karama and Adalci bread. Each bread was kept sealed and exposed for 7 days at different locations under ambient environmental conditions of Relative humidity and room temperature and observed daily for spoilage. The organisms obtained to be associated with the spoilage of bread were *Aspergillus niger*, *Mucor racemosus*, *Aspergillus fumigates*, *Aspergillus flavus*, *A. terreis*, *Cephalosporium* species, and *Paecilomyces* species. Percentage of occurrence varies from 17.30% to 8.75%. Since these fungi are known to produce aflatoxin. However, from the data and results obtained in this research, bread was shown to be seriously contaminated after 7 days and is unfit for human consumption as a result of the growth of these toxic producing different fungal species due to improper storage facilities and which would cause toxin which can cause serious health problem to human beings and even animals when consumed.

**Keywords:** Kampala bread, Adalci bread, Karama bread, fungi and Birnin Kebbi

### INTRODUCTION

Most of our foods are excellent sources for rapid microbial growth. Food materials contain organic substances in plenty and sufficient amount of water, and they may be either neutral or slightly acidic in nature (Roessler and Ballenger, 2006). They are subjected to natural contamination by many different kinds of microorganisms, including pathogens. Metabolic activities of microbes alter the condition of food, resulting in its spoilage (Angela *et al.*, 2010). Several insects are also responsible for the spread of microbes to the food. In general, the keeping quality of food depends on the success of preventing the entry of micro-organisms and restricting their growth (Knigh and Menlove, 2006). Foods are not only nutritious to consumers but are also an excellent source of nutrients for microbial growth. Depending upon the microorganism present, foods may spoil or be preserved by fermentation (Membre *et al.*, 2001)..

Bread is a food produced from wheat flour or other composite flours made of wheat and other cereal/crops such as cassava and maize at varying ratios. The dough formed is allowed to ferment and then baked in an oven. Bread is a very important source of nutrients as it contains proteins, carbohydrates, fats, various vitamins and minerals (Kent, 1981).. Enriched bread ranks as an energy food and as a source of many nutrients (Hoseney, 2008). However, various microorganisms are found to cause the spoilage of bread, especially the moulds. Mould spoilage of bakery products is a serious and costly problem for bakeries and the use of preservatives is, therefore, an attractive means to diminish spoilage and ensure safety (Smith, 1993). According to the previous studies (Banwart, 2004), bread

moulds like *Mucor* and *Rhizopus* are found to grow first during bread spoilage. This is followed by some other fungi like *Aspergillus*, *Penicillium* and *Fusarium* sp. Among this *Penicillium* sp is the most common one though *Aspergillus* sp may be of greater significance in tropical countries (Legar, 1993). Therefore, this study was designed to enumerate fungi populations in three (3) different bread samples namely; Adalci, Kampala, Karama through isolation and identification.

### MATERIALS AND METHODS

#### Study Area

The study was conducted in Kebbi State, Nigeria. Kebbi State is located between latitude 10°N to 13°N and longitude 3°E to 6°E in North-Western Nigeria with its capital at Birnin Kebbi. It borders the Sokoto State to the north, Niger State to the south and Zamfara State to the east. The natural vegetation of the State comprises Sudan Savanna in the north and Northern Guinea Savanna in the South and Southeast (Nanoh and David, 2014).

#### Sample Collection

The 9 loaves of bread used in this study were purchased from different shops in the local market.

The samples collected were brought in sterile polythene bags to the laboratory for analysis. These were exposed to the laboratory environment for 7 days.

#### Sterilization of the Materials

All glasswares such as Petri-dishes, test tubes, conical flasks, were washed thoroughly with detergent and sterilized by dry heat in a hot air oven at a temperature of 160°C held for 45-60 min. Cooling time was allowed to

enable the items in the oven to cool slowly. The oven door was not opened until the temperature inside the oven has fallen to below 50°C, to avoid cracking of glassware and air containing contaminated organism being drawn into the oven (Cheesbrough, 2002).

#### Preparation of Potato Dextrose Agar Medium

Potato Dextrose Agar (PDA) was used. Its preparation was according to the manufacturer's specification and instruction. Streptomycin was incorporated used to prevent the growth of bacteria. Poured Petri dishes were left overnight to solidify and be certain of those free of contamination (Cheesbrough, 2002).

#### Mould Counts and Isolation

Twenty gram (20g) of bread was homogenized in 180ml of sterile distilled water. The homogenate was serially diluted and 0.1ml of the selected dilutions were plated out on Potato Dextrose Agar (PDA) using the method as described by Cheesbrough (2002). The plates were incubated at 28°C ± 2°C for 24-72 hours. After incubation, discrete colonies were counted and their numbers noted. This was followed

by the isolation of the organisms into pure cultures on Potato Dextrose Agar (PDA). Each isolated was stained with lactophenol cotton blue and identified based on their morphological features, spores, and hyphae (Beauchat, 1992).

#### Determination of Frequency of Occurrence

The frequencies of occurrence of fungi associated with bread samples were determined using the formula. %Frequency= Number of the identified isolate/Total number of plates x 100. The data obtained were presented as percentages using SPSS (Statistical Package for the Social Sciences).

#### RESULTS

The results obtained in Table 1 showed the occurrences of fungi and percentage distribution of fungi isolated (Table 2) showed that Adalci bread had the highest percentage of fungi (17.30) while some species were not detected as seen in Table 1 respectively. Table 3 shows the identification of the isolate based on cultural characteristics, colony morphology (cell size, shape, pigmentation and arrangement).

**Table 1:** Occurrences of fungal species in the three (3) different samples

Organisms isolated	Kampala bread	Karanci bread	Adalci bread
<i>Aspergillus niger</i>	+	-	+
<i>Aspergillus fumigates</i>	+	+	-
<i>Aspergillus terreus</i>	-	+	+
<i>Mucor</i> species	+	+	+
<i>Paecilomyces</i> species	+	-	+
<i>Cephalosporium</i> species	+	-	-
<i>Aspergillus flavus</i>	+	+	+
<i>Penicillium</i> species	-	+	+

Key: - = Not detected, + = Detected

**Table 2:** Percentage distribution of Isolated fungal species

Isolated fungi	Kampala bread	Karanci bread	Adalci bread
<i>Aspergillus niger</i>	15.75	-	17.30
<i>Aspergillus fumigates</i>	14.01	12.32	-
<i>Aspergillus terreus</i>	-	10.30	13.46
<i>Mucor</i> species	15.00	15.40	15.38
<i>Paecilomyces</i> species	10.50	-	11.50
<i>Cephalosporium</i> species	10.11	-	-
<i>Aspergillus flavus</i>	12.34	13.00	9.65
<i>Penicillium</i> species	-	8.50	7.69

**Table 3:** Morphological microscopic examination of fungi isolates from the bread

Colony appearance	Microscopic observation
<b>Compact white yellow</b>	A dark dense conidial head are seen with bi-seriate, borne on brown often septate <i>Aspergillus niger</i>
<b>Dark green in colour</b>	Colonies consisting of dense short conidiophores with aerial hyphae Smooth walled <i>Aspergillus terreus</i>
<b>Pink im color</b>	Velvety colonies, hyphae septate conidiospores borne laterally on hyphae non septate with numerous sterigmata conidia borne in chains on sterigmata <i>Aspergillus flavus</i>
<b>Whitish cottony, colony later becoming brown</b>	Branched short sponrongiospore are seen with casted walls <i>Mucor</i> species Septate hyphae with hyaline are formed with phialides directly on hyphal tips <i>Paecilomyces</i> species
<b>Grey white later becomes pink in color</b>	Hyphae were septate with conidiospores are thin ramified the septate borne vertical <i>Cephalosporium</i> species
<b>Greenish white colony</b>	Highly branched multinucleated septate with numerous conidiophores sprout on mycelis bearing conidiospores individually <i>Penicillium</i> species

## DISCUSSION

Spoilage of bread by fungi may be due to post-processing contamination. Bread loaves fresh from the oven are free of molds or mold spores due to their thermal inactivation during the baking process (Ponte and Tsen, 1990). Bread becomes contaminated after baking, from the mold spores present in the atmosphere surrounding loaves during cooling, slicing, packaging, and storage (Keta, 2017). The results obtained from this study showed that four *Aspergillus* species namely; *A. niger*, *A. flavus*, *A. fumigatus*, *A. terreus*, and other species; *Mucor* species, *Paecilomyces*, *Cephalosporum*, and *Penicillium* sp. were isolated and identified with varying percentage as the sample differ. This was in conformity with the findings of Samson *et al.* (2007) who reported similar results. The predominant molds in bread and are responsible for its spoilage after the bread is allowed without preserved in refrigeration for 3 weeks. This is in agreement with the findings of Abellana *et al.* (1997b). *Aspergillus* spp. are commonly responsible for the black and light green colors often observed on spoilt bread.

The contamination of bread by fungi could also be as a result of poor handling practices in the food supply chain, storage conditions, distribution, marketing practices, and transportation. This study showed that the mold growth increased with the No of days. It depends on storage conditions. The isolated molds species obtained in this study are known to cause disease of various kind as reported by Zhao *et al.* (2008). The most common forms of bread deterioration are moisture loss and microbiological spoilage. Spoilage of bread and other bakery products by colonization and growth of fungi represents more than 90

percent of the total microbial contamination. However, a reduction in the dose of preservatives used to control mold spoilage in bakery products may lead, under certain conditions, to a reduction in the shelf-life of the product (Magan and Aldred, 2006).

## CONCLUSION

Since bread have been very important in human nutritional qualities, this then calls for proper handling of the produce from the production to consumption. All the breads are advised to be eaten new bread in either to avoid long term exposure to spoilage. Finally, the bakery who produce breads into bags for transportation, the marketers and consumers should take necessary precautions in preventing contamination and also try to create an environment that would discourage the growth or multiplication of microorganisms. All the above will help to reduce the risk of poisoning by aflatoxin and other mycotoxins which are normally produced by those fungi isolated in this study. Hence, this is a great concern to all.

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