

**COMPARATIVE ANALYSIS OF FUNGAL GROWTH ON BUTTERED AND
UNBUTTERED BREAD**

BY

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CERTIFICATION PAGE

This research project titled; COMPERATIVE ANALYSIS OF FUNGAL GROWTH ON BUTTERED AND UNBUTTERED BREAD by (DANJUMA ZAINAB 1910302273) has been read and approved by the undersigned as meeting the requirement for the Award of Bachelor of Science (Hons.) Degree in Biology in the Department of Biological sciences, Usmanu Danfodiyo University, Sokoto and is approved for its contribution to knowledge.

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DEDICATION

This project is dedicated to almighty Allah (s.w.t), who made it possible and my wonderful parents for their financial and moral support throughout my study period and also praying for my success. May Allah's infinite mercy and blessing continue to be with them (Amen).

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ABSTRACT

Bread is one of the most consumed staple foods in most countries and cultures. It is a good source of nutrients such as micronutrients and macronutrients that are essential for human health. In industry, bread possesses a special position both for processing and use as contrasted with other bakery products. The principle elements of bread are flour, water, salt, sugar, shortening and yeast. This study was carried out to isolate and identify fungi that is associated with the spoilage of buttered and unbuttered bread sold in Sokoto metropolis. Two bread samples were purchased at Kure Market (Buttered) and Sokoto State University (Unbuttered). Both samples were purchased and transported to mycology lab for analysis. 5.85g of Potato Dextrose Agar (PDA) and 0.1g of streptomycin was weighed and diluted with 150ml of distilled water, melted and Autoclaved at 121°C then timed to 15 minutes before bringing out the media. The fungi isolated were *Aspergillus niger*, *Aspergillus flavus*, and *Mucor racemosus*. *Aspergillus niger* (47.4%), *Aspergillus flavus* (34.2%) have the highest level of occurrence while the *Mucor racemosus* (18.4%) have the least level of occurrence. Therefore, the study recommended that measures as good hygiene in the bakeries and if necessary complementary post packaging heat treatments or modified atmosphere packaging is the best alternative.

CHAPTER ONE

1.0

INTRODUCTION

1.1 Background of the Study

Bread is a staple food in developed and developing world. It is widely accepted and considered a poor man's diet or sick man's diet. It is available in different forms and consumed in different ways. It can be taken alone as snack or with hot beverages (tea, cocoa, and coffee), cold nourishing drinks (chocolate drinks, milk, shakes etc.) and refreshing drinks (fruit drinks, fruit juices and carbonated drinks). Bread, may also be consumed with breakfast cereals such as corn and millet porridge, 'tom brown', etc (Adebayo *et al.*, 2016). The history of bread, although a bit controversial, is traced to ancient Israelites living in Egypt about 12,000 years ago or more (Zain *et al.*, 2021). Baking of bread in Nigeria started in Lagos around 1900 when slaves freed from Brazil and West India established bakery in the country (Babalola-Alao, 2018). Presently, bread is the second most consumed non-indigenous food in Nigeria after rice (Alexander, 2019).

Bread as a form of bakery product is a source of nutrients, such as micronutrients and macronutrients that are essential in human health (Potter and Hotchkiss 2006). A loaf of bread is a good source of carbohydrates, protein, fat, minerals, and vitamins essential for nourishment of the body and vitality. Bread is rich in fiber and folic acid. The process of producing bread involves mixing, kneading, proofing, shaping and baking (Balarabe, 2017). Mixing involves adding flour, salt, yeast, water, fats, sugars and other

ingredients to make a dough. The dough is made in such a way that will rise easily and be able to give bread a good quality to the consumer. Yeast is used in the dough which releases Carbon dioxide and the bread becomes spongy. Kneading by hand or machine is important for fermentation and helps to distribute temperature more evenly. The step where the dough rests and rises for the final time before baking is known as proofing also known as proving. Shape the dough into desired form, preheat the oven, prepare it for steaming and baked the bread.

Fungi are member of the group of organisms that includes microorganism such as yeasts, molds and mushrooms. They are eukaryotic organisms that are either plant, animals or protists. They are composed of filament called hyphae, their cells are long and thread like and connected end to end (pelczaret *al.*, 2003). The disciple of biology devoted to the study of fungi is known as mycology. Fungi play an important role in ecosystem, such as breaking down organic matter and acting as decomposers which make them saprophytes, and they can also be parasites on plants and animals. Fungi are usually classified in four divisions these are, ascomycota (yeast and sac fungi), chytridiomycota (thytrids), zygomycota (bread mold), and basidiomycota (club fungi). There are also two conventional groups which are not recognize as formal taxonomic groups. There are deuteromycota and lichens (Allexoprillos, 1962).

The growth of mold in bread is a substrate-saprophyte relationship with the bread providing nourishment and the mold surviving on it as long as favorable environmental conditions prevail. Mold growth in bread is also favored by their ability to synthesize

proteolytic and amylolytic enzymes (Baur, 2001). Furthermore, moisture condensation on a bread surface due to packaging while the product is not completely cool, may be conducive to mold growth (Marin *et al.*, 2002). A major cause of growth of mold in bread is long storage, resulting in the growth of molds which may produce toxins which lead to food poisoning when such foods are consumed (Vytrasova *et al.*, 2002).

1.2 Statement of the Research Problem

A variety of bakery products are available in the market. Bread which has now become the essential food item for a vast majority of the population (John and Mishra, 2017). There are different types of bread some which are buttered (which is made of butter) and unbuttered (which is made without butter). Even when the bread is prepared under a standardized environment and condition, it is often prone to microbial spoilage. Although baking temperature destroys fungal spores and other microorganisms associated with bread dough, post-processing contamination is quite unavoidable, mostly in developing countries. Post-processing contamination of bread is usually from mold spores present in the atmosphere surrounding loaves during cooling, slicing, packaging, and storage. The common source of microbial spoilage is due to mold growth. Microbial spoilage of bread like other bakery products depends on the water activity and the relative humidity of the environment. Bread stored under low relative humidity is less susceptible to microbial spoilage (Saranraj and Geetha, 2012). According to previous studies (Amadi *et al.*, 2014) bread molds like *Mucor* and *Rhizopus* are found to grow first during bread spoilage. This is followed by some other fungi like *Aspergillus*, *Penicillium*, and *Fusarium* (Banwart, 2004).

1.3 Justification of the Study

Mold spoilage of bakery products like bread 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). Preservation of bread also entails the use of modified atmosphere packaging, freezing, ultraviolet irradiation, pasteurization among others (Shahnwaz,2012). The effectiveness of chemical preservatives is dependent n water activity, composition, storage, pH and temperature of the product. It is well to point out immediately, that fungi are but one of several major reasons for the spoilage of bread (Sharma, 1992). Bread spoilage is a big financial loss to bakers and vendors. The health of the general public is at risk when unwholesome bread is sold by some vendors This research was carried out in order to find control measures to the fungi associated with the spoilage of buttered and unbuttered bread sold in Sokoto metropolis and to know which of the breads is more prevalent.

1.4 Aim and Objectives of the Study

The aim of the study is to assess the fungal on buttered and unbuttered bread. While the objectives were to:

- i. Isolate and identify fungal organisms associated with bread spoilage.
- ii. Know the prevalence of this fungi infestation on bread samples.

CHAPTER TWO

2.0

LITERATURE REVIEW

2.1 The Biology of Fungi

Fungi is the plural word fungus which is derived from the latin word fungour. Fungi are diverse group of eukaryotic organism with an estimated number of species in the range of 2-3 million (Nalin *et al.*, 2024). They are primarily heterotrophs that occur as unicellular to multicellular organisms and are vital in ecosystem functioning (Hyde *et al.*, 2018). They are made up of threadlike structures called hyphae, which collectively form a network called mycelium. The mycelium is the main body of the fungus and is responsible for nutrient absorption. They can either be yeast, or mold. Almost all the fungi have a filamentous structure except the yeast. The branch of biology that deals with the study of fungi is called mycology and the scientist who is concern with the fungi is called mycologist. Fungi reproduce through both sexual and asexual means, varying among different fungal species. The sexual mode of reproduction is referred to as teleomorph and the asexual mode of reproduction is referred to as anamorph. Asexual reproduction involves the formation of spores, which can be produced externally or within specialized structure called sporangia. These spores are then released into the environment where they germinate under suitable conditions to form new fungal organisms. Some prominent examples of asexual spore-producing fungi include *Aspergillus* and *Penicillium*. Sexual reproduction in fungi typically involves the fusion of specialized haploid cells called gametes. This process often leads to the formation of

zygotes, which then develop into new fungal structures. Notable examples of fungi that reproduce sexually include mushrooms (Basidiomycota) and molds (such as *rhizopus*).

Fungi exhibit various nutritional strategies. Saprophytic fungi obtain nutrients by decomposing and absorbing organic matter from dead and decaying organisms. They play a crucial role in ecosystems by recycling nutrients. These fungi secrete extracellular enzymes such as cellulose, proteases, and lipase that break down complex organic matter into simpler compounds. They then absorb these simpler molecules through their cell walls (Deacon, Jim, 2006) common saprophytic fungi include *penicillium*, *Rhizopus*, and *Aspergillus*. Fungi also exhibit parasitic and symbiotic (Mutualistic) mode of nutrition. They engage in symbiotic relationships where both partners benefit. One form of mutualism is mycorrhiza, where fungi and plant roots interact. In mycorrhizal associations, fungi extend the root surface area allowing for better water and nutrient absorption (notable phosphorus). In return, the plant supplies the fungus with carbohydrates produced during photosynthesis (Moore *et al.*, 2011). Lichen is a beneficial association between fungi and photosynthetic plants such as algae or cyanobacteria. The fungal partner, called the mycobiont, provides structure and protection, while the photosynthetic partner, called photobiont produces organic carbon compounds through photosynthesis, which the fungi utilize for nutrition. Lichen species are known with fungi from the Ascomycota and Basidiomycota phyla. Parasitic fungi derive nutrients by directly attacking living organisms, typically plants, animals, or other fungi. Parasites penetrate the host tissues and absorb nutrients directly from the cells. They

often cause diseases in their hosts. Examples are pathogenic fungi. They classified based on their reproductive structures and methods the main phyla of fungi include;

1. Phylum Ascomycota (Sac fungi)

They are also called sac fungi. They can be decomposers, parasitic, or saprophytic. They are characterized by budding or fussion as the primary means of asexual reproduction, and have sexual states that are not enclosed in fruiting bodies (Kurtzman *et al.*, 2011).

2. Phylum Zygomycota (Zygomycetes)

They are formed by the fusion of two different cells. Zygomycetes are characterized by their reproduction through the formation of zygospores. They include molds like bread molds.

3. Phylum Basidiomycota (club fungi)

Basidiomycetes or club fungi include mushrooms, puffballs, rusts and mostly live as saprobes, pathogens of plants and animals and ectomycorrhizal species (He *et al.*, 2022). Traditionally, fungi producing sexually by means of basidia but based on cultural, microscopic and molecular phylogenetic methods, several genera of basidiomycota also reproduce asexually (Hyde *et al.*, 2011).

4. Phylum Chitridiomycota (Chitrids)

Chitrids are primitive fungi that usually have flagellated spores called zoospores. Members of the phylum are reported from both aquatic and terrestrial ecosystems, mainly as saprotrophic, with many facultative and obligate parasites of algae (Galindo *et al.*, 2021).

5. Phylum Deuteromycota (fungi Imperfecti)

Deuteromycetes are diverse group of fungi that do not fit neatly into the traditional fungal classification system due to lack of a known sexual reproductive stage, they may include many common molds and pathogens.

2.2 Bread Mold

Bread mold grows profusely on wet slice of bread as opposed to on a dry slice of bread due to the fact microorganisms want ideal temperature and moisture to develop and if there may be no moisture to develop and if there may be no moisture, they will now no longer develop profusely. The contamination of food by fungi could also be as a result of poor handling practices, marketing practices and transportation. Bread mold is a kind of fungi that is commonly found on bread surface. It takes food and nutrients from the bread and causes damage to the surface where it lives, as well as bad taste to the bread (Anon, 2001). When bread is exposed in the environment, including the air in our homes, it can become a suitable substrate for fungal growth due to its high moisture content and nutrients. Fungi particularly mold species, may grow more rapidly and extensively on buttered bread compared to unbuttered bread due to the increased in nutrients such as butter which contain fats and proteins and moisture availability which create a more favorable environment for fungal growth.

2.3 Shelf Life of Bread

In the domestic environment and distribution chain, thermal abuse is a common thing. Usually in the kitchen, room temperature conditions defer extensively, this makes

controlling temperature in the domestic freezer and refrigerators very poor. Food manufacturers therefore have the mandate to understand the characteristics of products during storage and the fluctuating conditions that their products are likely to face in the supply chain (Adeniran and Ambiose, 2019). Health safety and ideal sensory properties are the main benchers used to assess the quality of bakery products. The resilience of bakery products with medium and high humidity content is usually narrowed by mould growth. Mode of product preparation, season and product type affects the growth of moulds in most products and these account for a percentage of loss between 1 and 5% in bakery products globally. According to Kuchenmuller *et al.*, (2013) after 48 hours of producing bread, it begins the spoilage resulting in substantial losses to the manufacturers. Mouldiness is among the main kinds of spoilage in bread due to microbes. These microbes can be prevented if the hygiene practices in bakeries are used in bread.

Al Mamun *et al.*, (2013) stated in their research that extending the shelf life of food has already been highlighted. Unit operations of food processing such as sterilization and pasteurization have been employed to achieve this. On the contrary, this cannot be applied in bakery products. Adding that bread is categorized as one of the foods which have a very short shelf life. Also stated that bread has two main structures which are the crust and crumb. The crust has a lower water activity and dry matter than the crumb thus it forms a protective primary barrier that prevents moisture loss and attack of microorganisms. The use of acceptable high temperature during processing and also new and quality technology such as the use of chemical additives and part baking could help avert spoilage of bread for an appreciable period of time. Water activity as stated by

Adeniran and Ambiose (2019) regulates the accessibility of water for the proliferation of microorganisms. The water activity value is solely dependent on the bread type, the time, the environmental conditions and the age of the bread. Vlasek *et al.*, (2013) stated that due to the composition and high-water activity, bread is known to be an appropriate substrate that promotes the growth of microorganisms such as moulds. Katkar (2011) reported that another possible factor affecting the shelf life of bread is humidity. A humidity is known to affect the crust colour and final quality of bread by increasing its moisture content and volume. Vlasek (2013) recounted that most of the studies done on the integration of humidity in the baking emphasize mostly on the characteristics of the product subsequently after baking. There is a gap however in the study of the outcome of baking and storing bread under a particular temperature and humidity proceeding to a shelf-life study.

2.4 Factors Influencing Fungal Growth on Bread

Fungi require a certain level of moisture to grow. Moist bread provides an ideal environment for fungal spores to germinate and multiply. A study by Pitt and Hocking (2009) demonstrated that higher moisture content in bread can lead to more rapid fungal growth. Temperature also plays a significant role in the growth of fungi on bread. Most fungi thrive at room temperature, but different species may have specific temperature requirements (Skovgaard *et al.*, 2016). Mold grows best in warm conditions, particularly in the range of 24degrees to 29degrees. Beyond this range, growth may slow down or stop. Refrigerating bread can significantly slow down mold growth, although it may also change the breads texture. The pH of bread can influence fungal growth. Fungi generally

prefer slightly acidic conditions (pH levels around 5 to 6). Previous research also state that pH levels can affect fungal colonization on different types of bread (Megan and Aldred, 2007). The ingredients used in bread can affect its pH. For example, a whole grain breads may have different pH levels compared to white bread. Some breads contain preservatives to inhibit fungal growth and extend shelf life. Available nutrients like sugars, proteins, and vitamins to can cause fungal growth. Stale bread with higher sugar content may be more prone to fungal infestation. Hygiene and Cross-Contamination: proper hygiene practices during bread handling and storage

2.5 Economic Importance of Fungi

Fungi mediate essential process within ecosystems such as nutrient cycling. Through their role in decomposition, fungi regulate soil fertility, making available minerals such as nitrogen, phosphorus, sulphur and carbon. They are the only organisms known to extensively degrade lignin, many fungi, through their assimilation of these substances, produce food in the form of edible mushrooms. In some instances, however, fungi facilitate mineral uptake in plants (more than 85% of vascular plant depend on this). This is nutrients would otherwise be leached from the soil. Many species of fungi are found to have antibiotic properties that are used in production of antibiotics (Ryan, 2004.) example penicillin is obtained from the species of *Penicillium*. Some fungi which are parasitic caterpillar have also been traditionally used as medicine (Bodey *et al.*, 1992). The Chinese have used a particular caterpillar fungus as tonic for hundreds of years (Anon, 2001). Certain chemical compounds isolated from the fungus may prove to be useful treatment for certain types of cancer.

Fungi are used in the production of various food products, such as bread, cheese, and alcoholic beverages. For example, yeast (a type of fungi) is used in the fermentation process for bread and beer production (Steinkraus, 1996). Bioremediation which involves the breaking down of pollutant and contaminants in the environment, making them valuable tools for cleaning up polluted site is also an economic importance of fungi (Verma, 2013). Fungi are also used in biotechnological processes for the production of enzymes, organic acids, vitamins, and biofuels. Fungal enzymes are widely used in industrial processes, such as the production of detergents and bioethanol (Polmeet *al.*, 2014).

CHAPTER THREE

3.0

MATERIALS AND METHODS

3.1 Study Area

This study was conducted in Sokoto, Sokoto state Nigeria. The state is geographically situated between longitude 11 30' to 13 50' East and latitude 4 to 6 North, encompassing a land area of 26,648.48 square kilometers. Sokoto State is bordered by Kebbi state to the south, Zamfara state to the east, and the Republic of Niger to the north. As of 2015, the state had an estimated population of about 4,742,459, with a population density of 95.9 persons per square kilometer and an annual growth rate of 3% according to the 2006 census (NPC, 2007). The local population is engaged in various activities including farming, trading, and commerce, with a significant number employed in both private and public sectors (MOCIT, 2002).

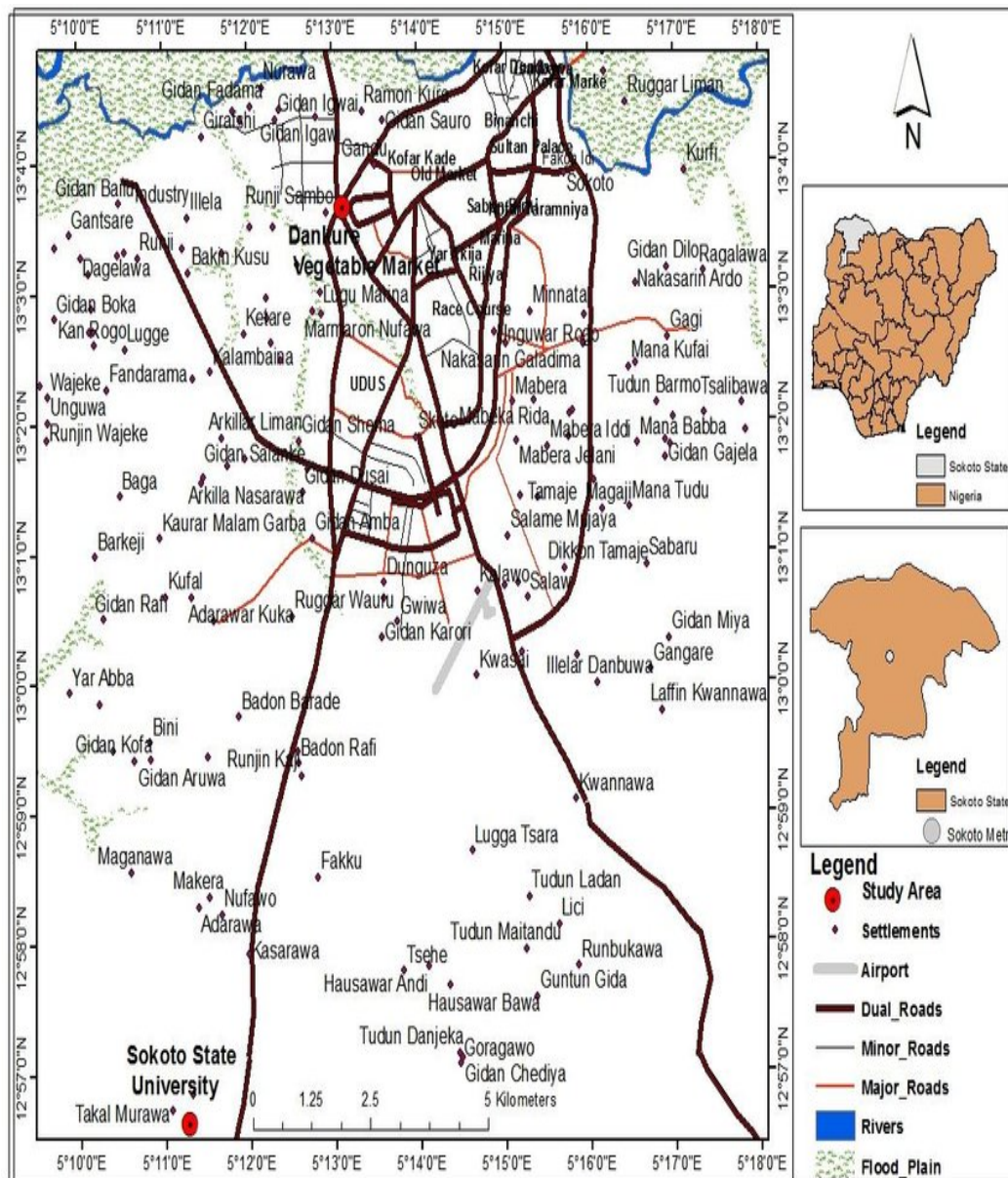


Figure 3.1 Map of Study area showing Sampling Location

3.2 Sample Collection

Two bread samples were purchased at Kure market (buttered) and Sokoto state university (unbuttered) within Sokoto metropolis. All the bread samples purchased were placed in sterilized polythene bags that have been labeled to indicate appropriately the buttered and unbuttered breads. The samples were then transported to the Mycology Laboratory in the Department of Biological Sciences, Usman Danfodiyo University Sokoto for analysis. The bread samples were kept in the mycology laboratory for some Days to allow the growth of fungi. After three (3) the buttered bread starts to develop fungal growth while the unbuttered bread takes five (5) days for the fungal growth. Both samples were kept for a complete seven (days) to enable the fungi to grow completely. Fungi grow faster on buttered bread compared to unbuttered bread due to added moisture from the butter.

3.3 Preparation of Culture Media

Hand gloves and face mask was worn before the preparation of the media. 5.85g of PDA (Potato dextrose agar) and 0.1g of streptomycin was weighted and transferred into a conical flask. 150ml of distilled water was measured using a measuring cylinder. The distilled water was then poured into the conical flask and shake for some minutes before heating. The conical flask was airtight and placed on a thermoplate, continue shaking it until all the particles melt. The conical flask was airtight with cotton wool and foil paper and then autoclave at 121°C then time to 15 minutes and allow the gauge to return back to zero (0). Allow the autoclave to cool down before bringing out the media in the conical flask so as to avoid burning your hand due to the heat.

3.4 Inoculation

The surface of the infected area was sterilized using ethanol before placing the petri dishes to avoid contamination. Six sterilized petri dishes were labeled according to bread samples three (3) each for buttered bread and unbuttered bread as the samples both produce three different colours of fungal growth such as green, black and white colour respectively. The prepared media in the conical flask after cooling down was carefully poured into labeled petri dishes then the mouth of the conical flask was heated with a Bunsen flame to avoid contamination. The media poured into the petri dish was allowed for 24 hours to solidify. After solidifying the spoiled bread samples were then inoculated into the petri dishes by taking different kinds of fungi according to the colour from the bread samples into different petri dishes that correspond to the labeling using an inoculating needle. After seven days four pure cultures and two impure cultures were observed. The pure cultures were stored in the refrigerator while the impure culture was sub-cultured (inoculated) to form a pure culture.

3.5 Incubation

Another six petri dishes were sterilized for incubation. A media with 5.85g of PDA () and 0.1g of streptomycin was weighed and transferred into the conical flask. 150ml of distilled water was measured using a measuring cylinder and all transferred into the conical flask for the preparation of the media. The media was prepared, autoclaved and cooled then transferred into the conical flask to solidify before the incubation. The impure cultures were then incubated at room temperature (27±3°C) for seven days (7) by sub-culturing different fungal species according to the colors in three petri dishes for each colour. During

the incubation period any sign of pure culture observed was noted and allow to have full growth.

3.6 Isolation and Identification

The colonies of different fungal species were identified based on morphological characteristics of individual species of fungi and then observed under microscope for microscopic characteristics. Microscopic identification was made by putting a drop of distilled water on a glass slide, a small portion of a fungal growth was transferred onto the distilled water on the glass slide and tease properly with teased inoculating needles. The slide was then covered with cover slip. The prepared slide was then examined systematically with aid of light microscope using x10 objective lens. The morphological characteristics, nature of mycelium, type of hyphae, spores and structures were used as the criteria for identification of fungal isolates.

CHAPTER FOUR

4.0

RESULTS

4.1 Fungi Isolated from Buttered and Unbuttered Bread

The table shown below shows the fungi isolated from both buttered and unbuttered bread purchased within Sokoto metropolis, three fungal species were isolated from both sampl. The fungi isolated from both buttered and unbuttered bread were *Aspergillus niger*, *Aspergillus flavus* and *Mucor racemosus*. The *Aspergillus niger* causes the black mold decay, *Aspergillus flavus* causes the green mold decay and *Mucor racemosus* causes the white decay.

Table 4.1 Fungi isolated from buttered and unbuttered bread

Organism	Buttered	Unbuttered
<i>Aspergillus niger</i>	10	8
<i>Aspergillus flavus</i>	6	7
<i>Mucor racemosus</i>	3	4
Total	19	19

Table 4.2 Frequency of occurrence of fungi isolated from buttered and unbuttered bread.

The table below shows the total number times this fungi isolates appears. *Aspergillus niger* and *Aspergillus flavus* have the highest frequency of 18 and 13 while *Mucor racemosus* have the lowest frequency of 7.

Table 4.2 Frequency of occurrence of fungi isolated from buttered and unbuttered bread

Organism	Frequency of occurrence
<i>Aspergillus niger</i>	18
<i>Aspergillus flavus</i>	13
<i>Mucor racemosus</i>	7
Total	38

Table 4.3 The percentage of occurrence of the fungi isolated from buttered and unbuttered bread.

The table below shows the frequency of occurrence of the fungal isolates divided by the overall total number of fungal occurrences multiply by 100 to shows the percentage of occurrence of fungi isolated from the samples. *Aspergillus niger* and *Aspergillus flavus* have the highest percentage frequency of 47.4% and 34.2% respectively with *Mucor racemosus* having the lowest percentage frequency of 18.4%.

Table 4.3 Percentage of occurrence of fungi isolated from buttered and unbuttered bread

Organism	Percentage of occurrence
<i>Aspergillus niger</i>	47.4%
<i>Aspergillus flavus</i>	34.2%
<i>Mucor racemosus</i>	18.4%
<i>Total</i>	100%

Table 4.4 Morphological characteristics and identities of the fungal isolates. The isolates are identified has *Aspergillus niger*, *Aspergillus flavus* and *Mucor racemosus*. Table table below shows the characteristics, structure, distribution, physical distribution, of the fungi isolates.

Table 4.4 Morphological characterization and identification of fungal isolates

Organism	Colony character
<i>Aspergillus niger</i>	Colonies of <i>Aspergillus niger</i> consist of a compact white or yellow basal felt with dense layer of dark brown to black conidiophores. Hyphae are usually septate. They have a worldwide distribution and mostly growing as saprophyte its optimal growth temperature is 20-40°C with good growth at 37°C.
<i>Aspergillus flavus</i>	They are commonly powdery masses of yellowish-green conidophores on the upper surface and reddish-gold on the lower surface. The yellowish green later becoming dark yellow green. Hyphae growth usually occurs by thread-like branching and produce mycelia, hyphae are usually septate. It can grow between 17-19°C and 47-48°C with optimalteperature between 25 and 42°C.

Mucor racemosus

Whitish cottony, colony later becoming brownish-gray.

It consist of tall and short sporangiophores.

Sporangiophores branched (in a mixed sympodial and monopodial way), the short branches sometimes recurved, with encrusted walls. World-wide distribution

in soil, dung, food, seeds of wheat, barley, rice, tomatoes, groundnuts, tea, and animals. Good growth and sporulation is at 5-30°C with optimum temperature of 20-25°C and maximum temperature of 37°C.

CHAPTER FIVE

DISCUSSION

The result obtained from this findings shows that both buttered and unbuttered bread are contaminated with spores after the slice were placed in a dark, humid environment which stimulate the fungal growth. Three different species of fungi were discovered from both samples examined. *Aspergillus niger*, *Aspergillus flavus* and *Mucor racemosus* were recovered with varying percentage on both buttered and unbuttered bread. The buttered bread slice showed a more rapid growth of fungi compared to unbuttered slice due to varying nutrient compositions of the two substrates. Different fungi species have specific requirements for growth and the presence of butter on the buttered bread favored the growth of certain fungi over others.

This findings support earlier evidence that foodborne illness are prevalent worldwide and contribute to approximately one-third of global mortality (Khanom *et al.*, 2016). Bread's nutrient-rich composition fosters an environment conducive to microbial growth and metabolism, making it perishable. This creates the potential for harmful microorganisms like *Aspergillus* and *Mucor* to persist within the bread matrix, leading to spoilage and rendering the product unsuitable for consumption. Bread mold is so common because of the ingredients in bread are excellent source of nutrition for many molds to grow and thrive. It also contains limited moisture content, which is why mold can grow so well instead of bacteria or yeast that requires higher moisture levels to survive (Abigail, 2012). Bread is prepared by baking a dough made of flour (groundgrain) and water, and often

other ingredients. It may be leavened or unleavened. Salt, fat, and a leavening agent such as yeast are common ingredients, though breads may contain a range of other ingredients (Jacob, 2012). Most commercial loaves on the market today contain calcium propionate to inhibit the growth of fungi. The shelf life of most bakery products is often affected by moulds such as *Aspergillus niger*, *Aspergillus flavus* and *Mucor racemosus*.

The fungi play a key roles during the spoilage of foods that are high in moisture. An acidic pH between 3.5 and 5.5 tend to favor the growth of moulds. This further implies that moulds are more susceptible to cause spoilage of foods with a pH less than 4.5 as compared with bacteria (shepherd, 2018). Bread has a pH range of 4.0-5.8 making it susceptible to mould spoilage. According to savin, (2022) reducing storage temperature from 27°C to 21°C could considerably reduce mould growth and double the shelf life of bakery products

Implementing hygienic processing practices is essential to mitigate the risk of contamination and food poisoning, safeguarding consumers health (Hathout and Aly, 2014). Addressing the issue of unhygienic practices in bread production is of paramount importance. For instance, bread vendors and hawkers may inadvertently contaminate the equipment used in bakeries and points of distribution, allowing contaminants to enter the bread during various stages of handling and transportation (Jard et al., 2021). By proactively addressing unhygienic practices and adopting stringent control measures, the risk of contamination and associated health hazards can be significantly reduced, safeguarding consumers and maintaining the quality and safety of bread products (Amadi

et al., 2014). Utilization of preservatives has emerged as an alternative approach to extend shelf life of bakery products despite not being favored by consumers. So, preservatives can play a big role in how quickly bread molds grow whether on white or wheat (Lynn, 2013). Commonly employed in the baking industry are propionates, sorbates, and sometimes benzoates (Hassan *et al.*, 2014). However, despite their effectiveness, certain fungal species have displayed resistance even at the permissible maximum concentrations allowed in food. Another crucial measure to prevent fungal spoilage is adopting a hygienic layout for buildings and facilities. This includes segregating post-baking areas (cooling and packing) from the bread making processing area, thereby reducing the influx of spores from raw materials that may contaminate freshly baked slices (Shephard, 2018). Additionally, minimizing the time of cooling and packaging can positively impact shelf life. By reducing the exposure of bread to contaminated air, the chance of spore deposition on the product surface is diminished, leading to an extended shelf life of bread.

CONCLUSION

Our study findings revealed that bread samples distributed at Kure Market and Sokoto State University exhibited contamination with a diverse array of fungal species. This contamination poses a significant health risk to human health, potentially exposing the population to food-borne diseases. Bread is abundant in carbohydrates, serving as a primary energy source for the body. Moreover, it acts as a crucial fuel for essential organs such as the brain, kidneys, heart muscles, and central nervous system. Additionally, the presence of such contaminants indicates inadequate hygienic practices carried out by the handlers involved in the bread's production and distribution.”

RECOMMENDATIONS

Moulds are responsible for spoilage of bread and baked products. Based on the finding of this study the following recommendations were made;

1. Frequent and sufficient hand washing of the hands of individuals involved in food handling.
2. It is recommended to buy bread from reputable and approved sources that adhere to proper processing and packaging.
3. Do not wrap hot bread products as it causes condensation status within the wrap leather which accelerates mould growth.
4. If mould colonies appeared, dispose the product, do not consume it, all or in part as some fungi can penetrate the bread to some depth which cannot be seen with the naked eyes.
5. Those selling bread who are mostly local women, villagers and children should be enlightened on the dangers of contamination.

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