TURKISH REPUBLIC OF NORTH CYPRUS

NEAR EAST UNIVERSITY

INSTITUTE OF GRADUATE STUDIES

Fungi Associated With The Spoilage Of Bread In Monrovia, Liberia

KAY S. DUNAH

THESIS FOR MASTER OF SCIENCE

Medical Microbiology And Clinical Microbiology Program

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Prof. Dr. H. Kaya Süer

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APPROVAL

This thesis was approved by the Near East University Postgraduate Study-Education and Examination Regulations, as well as the thesis committee and Board of Directors.

Examining Committee — Signature

Head of the Committee: Prof.Dr.H.Kaya SÜER .....

Member: Assoc.Prof.Dr.Ulaş HURDOĞANOĞLU .....

Member: Assoc.Prof.Dr.Ferdiye TANAR .....

Supervisor: Prof.Dr.H.Kaya SÜER .....

Approved by the Head of the Department

04../.03./2024

.....

Assoc.Prof.Dr.Emrah RUH

Head of the department

Approved by the Institute of Graduate Studies

...../...../2024

Prof. Dr. Kemal Hüsnü Can BAŞER

Head of the Institute

DECLARATION

I declare that I am the person who wrote this thesis study and that I have approached every aspect of its execution, from planning to writing, with honesty and adherence to ethical standards. Every piece of data in this thesis was gathered in compliance with ethical and academic guidelines. Everything I said and did that wasn't directly related to this thesis topic has been appropriately cited and added to the list of references. In addition, I have not done anything throughout the course of this research or the preparation of this thesis that would breach copyright or patent rights.

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ÖZET

Bu araştırmanın amacı Monrovia, Liberya'da ekmeğin bozulmasından sorumlu olan mantar türlerini tespit etmektir. Aynı zamanda mantarlarla kontamine olmuş ürünlerin tüketilmesiyle ilgili tehlikelere ilişkin farkındalığın arttırılmasıdır. Kültür ortamı olarak Sabouraud dekstroz agar kullanıldı ve bu ortam kullanılarak mantarların ayrıştırılmasında yayma plak yöntemi kullanıldı. Numunelerin pH ve nem içeriği yüzdesi ölçüldü. Bu araştırmanın temel amacı ekmeğin bozulmasından sorumlu olan mantarları belirlemektir. Ekmek örneklerinin mantar sayıları 1,2x103 kob/ml ile 4,3x103 kob/ml arasında değişmektedir. Fula ekmeğinin sayısı 3,0x103 Cfu/ml, Fanti ekmeğinin sayısı 1,2x103 cfu/ml ve Lübnan ekmeğinin sayısı ise 4,3x103 cfu/ml idi. kısa ekmek ve Pirinç ekmeğinde sırasıyla 2,1x103 cfu/ml ve 2,2x103 cfu/ml mantar sayısı vardı.

Ekmek numunesinden elde edilen Aspergillus SPP, Rhizopus spp, Mucor spp, Penicillum spp ve Cladosporium spp'yi içeren mantarların görünür ve mikroskobik özellikleri incelenerek teşhis edildi. Bu mantarın ortaya çıkma sıklığını belirlemek için ekmek numuneleri analiz edildi. Örneklerin tamamında Aspergillus SPP'nin mevcut olduğu, Pirinç ekmeği hariç tüm örneklerde ise Rhizopus spp ve Mucor spp'nin mevcut olduğu belirlendi. Penicillium türleri fantain ekmeği ve Lübnan ekmeği dışında tüm ekmek türlerinde bulunurken, Cladosporium türleri Fula ve Pirinç ekmeklerinde bulunmaktadır.

Mevcut nem seviyesi %44,2 ila %44,7 arasında değişirken pH 5,90 ila 5,98 arasında değişir. Fanti ekmeğinin nem oranı %44,2, pH değeri ise 5,98 olarak ölçüldü. Lübnan ekmeğinin nem içeriği %44,7 ve pH değeri 5,90 idi. Kurabiyenin nem içeriği yüzdesi %44,3 ve pH değeri 5,9'du. Pirinç ekmeğinin nem içeriğinin %4,44, pH değerinin ise 5,95 olduğu belirlendi. Fula ekmeğinin nem içeriği %44,5, pH değeri ise 5,94'tür.

Contents

CHAPTER ONE

1.0 Introduction

This chapter examine extensively the background of the study, objective of the study, statement of the problems, research question, scope of the study significance of the study, Limitation of the study and operational definitions of variables and measurement.

1.1 Background of the study

Bread is a very good for human consumption. It also plays a major importance in our diet. It was originated dating back to the early ages. To this day, it remains one of the most widely consumed food products worldwide. (Oluwajoba et al., 2012).

Bread dough is formed by the combination of water and flour, along with several other ingredients, resulting in a diverse range of bread varieties.
Bread is a significant dietary source of essential nutrients, including carbs and minerals (Saranray and Geetha, 2012).

Bread and other bakery goods offer essential nutrients for daily activity, constituting approximately 50% of the required daily caloric intake. However, the preparation and management of bread in developing and poor countries, particularly in Liberia, West Africa, is a significant issue. Although the bread is being baked in a well-structured environment, it remains vulnerable to spoiling. While baking temperatures can help eradicate fungal spores, it is still difficult to completely avoid post-processing contamination, especially in a developing country like Liberia (Khanom et al., 2016).

Bread is typically initially contaminated by airborne dust particles. The spoilage of bread may be attributed to the water activity in the surrounding environment.

The spoilage of bread in Monrovia, Liberia poses a significant difficulty not just to a small number of daily consumers, but also to the entire health sector of Liberia and the surrounding sub-region of Africa. Bread is susceptible to physical, chemical, and microbiological deterioration. The proliferation of mold is a significant factor in the importance of bread and can occasionally be a significant challenge for bakeries.

Rhizopus sp, Penicillium sp, and Aspergillus sp are fungal species responsible for the deterioration of bread. Rhizopus stolonifer, which is known as bread mold, is one of the most prevalent types of molds.

Ropiness, a bacterial infection, is a significant cause of bread spoiling, second only to mold. It is more prevalent during the summer months, when the environmental conditions are conducive to bacterial growth. Bacillus subtilis is the primary cause of ropy bread. The growth of rope in bread can lead to spoilage and pose a health risk. The consumption of stale bread has been linked to cases of food-borne disease Ybar et al., 2012 and Rumeus and Turtoi, 2013.

1.2 Statement of the problem

Spoilage of bread is an epidemic in the sub-region of Africa mainly in Monrovia, Liberia. Despite awareness created on the safe guideline of the preparation and preservation of commercial bread, fungi spoilage of bread is a major health problem especially for those daily consumers.

This study has been done to identify and analyze those microorganisms that are associated with the spoilage of bread in Liberia.

1.3 Objective of the Study

The aim and objectives of this study are:

1. To identify the microorganisms associated with the spoilage of bread.
2. To determine the rate of fungi growth correlated with bread.
3. Characterize the fungi spoilage based on highest and lowest fungal counts.
4. To identify the PH levels and the percentage of moisture content of bread samples

1.4 Research Questions

To ascertain the microorganism responsible for bread deterioration in Monrovia,, Liberia the following questions are to be considered.

1. What is the growth rate of fungi associated with bread?
2. What is the factor influencing the spoilage of bread
3. What are the fungi that causes spoilage in bread?

1.5 Significance of the study

The significance of this study lies in its aim to identify the specific microorganisms (fungi) responsible for bread spoilage, the methods employed to identify them, the pH levels of the collected samples, and the characterization and identification of the fungi with the highest and lowest counts. The goal of this study is to raise awareness about the health risks associated with consuming spoiled bread**.**

1.6 Scope of the study

The investigation into this study was for the period of two months (November to December 2023). Analysis was done extensively on the said topic. This research will lead other researcher in surfing the internet, Libraries, clinical laboratory and bakeries to collect data for experimental purposes.

Samples of bread are FULA BREAD, FANTI BREAD, LEBANESE BREAD, SHORT BREAD and RICE BREAD

Limitations of the study

The study is an investigation into mentioned topic above. However, there were some major constraints that this study faced with. These constraints include time factor, the availability of some equipment for the laboratory experiment and honorary fees for the laboratory technician facilitating this research.

1.7 Operational Definitions of Variables and Measurement

The below listed words and phrases are used in this study, this term defines to provide readers with better understanding of the study.

1. Fungi – any group of spore-producing molds, yeast, mushrooms, and toadstools.
2. Spoilage – The process spoiling,
3. Identification – the action or process of identifying (locating) someone, something, or fact of being identified.
4. Bread – a staple food for human consumption.
5. Fula bread – as known in Liberia is a locally made bread with high texture usually made by the Fula tribe.
6. Fanti bread – as known in Liberia is a locally made bread with much softer texture and it is dominantly made by the Fantain tribe and sometimes made by the Fula tribe also.
7. Lebanese bread – a newly type of bakery bread in a round form. It is another soft texture bread made by Liberian for consumption.
8. Short bread – is a tradition bread usually made with white sugar, butter and plain wheat flour.
9. Rice bread – is a type of bread made from rice mix with flour and banana rather than white flour.

CHAPTER TWO

PREVIEW OF RELATED LITERATURE

2.0 Overview of chapter two

This chapter provides a comprehensive overview of the relevant literature and offers a detailed explanation of the microorganisms responsible for bread deterioration, as well as the measures taken to control them.

The presence of post-processing contamination has resulted in the development of mold and subsequent rotting of the bread. Initially, it was discovered that molds, specifically Rhizopus sp and Mucor sp, were primarily responsible for the spoilage of bread.

2.1 Fungi

Fungi are complex multicellular creatures composed of eukaryotic cells. They are ubiquitous but primarily inhabit terrestrial environments, predominantly within plant matter or soil. A plethora of fungus induce ailments in humans.

2.2 Types of fungi

The manufacture of bread primarily involves the utilization of flour and water as the main ingredients. The dough is produced through the process of fermenting flour, which enables the cultivation of certain microorganisms within the appropriate parameters. Nevertheless, this fermentation process might result in spoiling and an excessive proliferation of bacteria, so impeding the dough's capacity to generate the required quantity of gas for leavening. There are two types of bread deterioration: moldiness and ropiness.

During the production of bread, it undergoes baking at a moderate temperature, hence diminishing the probability of microorganisms' survival. Contamination occurs solely during the cooling process, as well as during the subsequent packing and handling activities in the surrounding environment.Colonies of filamentous fungi consisting of several cells

2.3 Taxonomy of Fungi

The realm Fungi is classified into five primary phyla based on their method of reproduction or molecular data analysis. Fungi that are polyphyletic and reproduce asexually are categorized for convenience in a separate group known as a "form phylum." There is disagreement among mycologists over this system. Advancements In the field of molecular biology, the process of sequencing 18S rRNA, a kind of RNA, consistently reveals novel and distinct connections among different categories of fungi. The five phyla of fungi are the Chytridiomycota (Chytrids), the Zygomycota (conjugated fungi), the Ascomycota (sac fungi), Basidiomycota (club fungi).

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Chytridiomycota: The Chytrids

The sonlyclass within the Phylum Chytridiomycota is the Chytridiomycetes. They are the most rudimentary and essential group of Eumycota. Based on fossil data, it is evident that the earliest recognized Chytrids emerged during the late pre-Cambrian epoch, many years ago. Chytrids, like other fungi, the cell walls of these organisms include chitin. However, a specific subgroup of chytrids has the simultaneous presence of cellulose and chitin in their cell membranes. Most chytrids consist of individual cells, although only a little number of organisms could develop into multicellular creatures and hyphae, which are coenocytic, meaning they lack cell partitions. They produce reproductive cells called gametes and diploid zoospores, which move by means of a single flagellum.

Zygomycota:

Zygomycetes are a diminutive group of fungus that fall within the Phylum Zygomycota. Rhizopus stolonifera is a well-known mold which quickly spreads on the surfaces of bread, fruits, and vegetables. Many species are saprobes, deriving sustenance from decomposing organic matter; a small number are parasites, primarily targeting insects. Zygomycetes have a significant impact on the commercial sector.

The metabolic byproducts of certain Rhizopus species function as intermediates in the synthesis of semi-synthetic steroid hormones. Zygomycetes have a thallus made up of coenocytic hyphae, where the nuclei are haploid during the organism's vegetative stage. Fungi typically engage in asexual reproduction through the production of sporangiospores. The black spores are

contained within enlarged sporangia, which are found at the darkened tips of bread mold. Upon landing on an appropriate medium, spores undergo germination and generate a fresh mycelium. Sexual reproduction is triggered when the environmental conditions become unfavorable. For gametangia to be generated and undergo karyogamy, two mating strains, one of type + and the other of type –, must be in close proximity. The maturing diploid zygospores possess robust exteriors that shield them from dehydration and other potential dangers. They can remain in a state of quiescence until the environmental conditions become favorable. After the zygospore germinates, it performs meiosis and produces haploid spores, which then grow into a new creature. The process of sexual reproduction in fungi is referred to as conjugation. However, it is important to note that it significantly varies from conjugation in bacteria and protists. Hence, these fungi are often recognized as "conjugated fungi."

Ascomycota

The taxonomic classification for the group of fungi commonly referred to as Sac Fungi is Ascomycota.

Most of the fungi that are acknowledged are categorized into the Phylum Ascomycota. The defining characteristic of this phylum is the presence of an ascus which is like a sac-like structure responsible for containing haploid ascospores. Numerous ascomycetes possess considerable economic worth. Specific organisms, such as yeasts used in baking, brewing, and wine fermentation, as well as truffles and morels, which are highly valued gourmet delicacies, have a beneficial function. Aspergillus oryzae is utilized in the fermentation of rice to produce sake**.** Furthermore, there are other types of ascomycetes that establish parasitic associations with plants and animals, including humans. For instance, individuals with AIDS who have a compromised immune system are at significant risk from fungal pneumonia. Ascomycetes not only physically invade and annihilate crops, but they also generate noxious secondary metabolites that render crops unsuitable for ingestion. Ascomycetes with filamentous growth produce hyphae that are divided into segments by porous septa, allowing for the movement of cytoplasm between neighboring cells. Conidia and asci, used for asexual and sexual reproduction respectively, are frequently separated from the vegetative hyphae by obstructed (non-perforated) septa. Asexual reproduction is widespread and characterized by the formation of conidiophores, which release haploid conidiospores. The sexual reproduction process initiates with the formation of distinct hyphae

originating from one of two distinct mating strains. The male variant of the bacteria forms an antheridium, which functions as a reproductive structure. In contrast, the "female" strain of the organism forms an ascogonium, which serves as a reproductive structure. During fertilization, the antheridium and the ascogonium undergo plasmogamy, a process in which their cytoplasm merges without nuclear fusion. Distinct ascogenous hyphae develop, wherein nuclei from both the "male" and "female" strains migrate together in pairs. Within each ascus, the nuclei of two or more haploid ascospores undergo fusion through the process of karyogamy. In the process of sexual reproduction, a large number of asci occupy a structure known as the ascocarp. Meiosis is the process by which the diploid nucleus produces haploid nuclei. Subsequently, the ascospores are liberated, undergo germination, and give rise to hyphae that are dispersed in the surroundings, initiating the development of fresh mycelia.

Basidiomycota:

With the aid of light microscope, the fungi classified under the Phylum Basidiomycota can be identified due to their distinctive club-shaped of the fruiting bodies known as basidia. These basidia are the enlarged end cells of a hypha. The basidia, the reproductive structures of these fungus, are frequently found within the recognizable mushroom, widely observed in fields following rainfall, on grocery shelves, and growing on lawns. These basidiomycetes that produce mushrooms are commonly Termed "gill fungi" because to the existence of gill-shaped projections on the lower surface of the cap. The term "gills" denotes densely clustered hyphae that provide a suitable environment for the growth of basidia. Additionally, this category encompasses shelf fungus, which adhere to tree bark resembling miniature shelves.

Furthermore, basidiomycota which are significant plant diseases, as well as toadstools and shelf fungi that grow in layers on tree trunks. The majority of edible fungus are classified under the Phylum Basidiomycota; yet, several basidiomycetes produce lethal toxins. As an illustration, Cryptococcus neoformans induces a serious respiratory disease. The majority of edible fungus are classified under the Phylum Basidiomycota; nonetheless, there are some basidiomycetes that produce highly toxic substances. One instance is when Cryptococcus neoformans leads to a serious respiratory disease. Mycelia from distinct mating strains can merge, the outcome is the creation of a secondary mycelium consisting of haploid nuclei from two distinct mating strains. The dikaryotic stage is the predominant and prominent stage in the lifecycle of basidiomycetes. In the end, the

secondary mycelium produces a basidiocarp, which is an aboveground reproductive structure. Commonly referred to as a mushroom.The basidiocarp facilitates the development of basidia, which are situated on the gills beneath its cap.

Deuteromycota,

The phylum known as the Imperfect Fungi comprises fungi that lack a sexual phase. Deuteromycota is a polyphyletic group, meaning that its species have closer evolutionary relationships with organisms from other phyla rather than with each other. Therefore, it cannot be classified as a real phylum and instead is referred to as a form phylum. Due to the absence of sexual features utilized for categorizing other fungi, their description is comparatively less comprehensive than other divisions. The majority of members reside on terrestrial habitats, while there are a few exceptions that inhabit aquatic environments. These organisms exhibit the growth of visible Mycelia has a velvety feel, generally referred to as mold. Based on molecular analysis, the ascomycetes are the most closely related group to the deuteromycetes. Indeed, certain species, such as Aspergillus, formerly categorized as imperfect fungus, are now classed as ascomycetes.

The Glomeromycota

A phylum that exclusively reside in close proximity to tree roots. The fossil records provide evidence of a lengthy evolutionary relationship between trees and their root symbionts. All members of this family exhibit arbuscular mycorrhizal symbiosis, in which the hyphae of the fungus establish a mutually beneficial relationship with the root cells. In this association, the plants furnish the fungus with a carbonaceous substrate and energy in the form of carbohydrates, while the fungus reciprocates by providing the plants with vital minerals obtained from the soil. Glomeromycetes lack sexual reproduction and are dependent on the presence of plant roots for survival. Despite possessing coenocytic hyphae similar to zygomycetes, they do not undergo zygospore formation. Based on DNA analysis, it has been determined that all glomeromycetes most likely originated from a single common ancestor, which classifies them as a monophyletic lineage (lumenlearning.com 2017).

2.4 Multicellular filamentous mold

Moulds consist of intricate filaments known as hyphae. Hyphae elongate at the apex and undergo repeated division along their entire length, resulting in the formation of extensive and intricately branched chains. The hyphae continue to proliferate and entwine with one another until they coalesce into a complex network of filaments known as a mycelium. Hyphal tips produce digestive enzymes. The enzymes in question degrade the organic stuff present in the soil into smaller molecules that serve as nourishment for the fungus.

A portion of the filamentous branches extend upwards and spores develop on these elevated branches. Spores are specialized structures that possess a protective layer, which serves to defend them from adverse environmental circumstances, such as desiccation and extreme temperatures. They are sufficiently little that a quantity ranging from 500 to 1000 might be accommodated on the surface area of a pin head.

Spores serve as reproductive structures for fungi, similar to how seeds function in plants. Spores are distributed by wind, rain, or insects. Ultimately, they settle in novel environments and, under favorable circumstances, initiate growth and generate fresh hyphae. Fungi, being immobile, employ spores to locate alternative habitats with reduced competition from other creatures.

2.5 The Organisms

The molds that are commonly found in the spoilage of bread are penicillium spp, even though Aspergillus spp. And Rhizopus spp. May be of greater significance in tropical region of the world. In wheat breads spoilage molds such as Cladosporium spp, Penicillium spp, Aspergillus spp, have been observed.

2.6 Filamentous fungus visible to the naked eye

Macroscopic filamentous fungus propagate by generating a mycelium beneath the surface. Mushrooms or toadstools, usually known as visible fruiting bodies, distinguish themselves from molds by their ability to produce spores. The fruiting body is composed of densely packed hyphae that undergo division to produce diverse elements of the fungal structure, including the cap and the

stem. The spores are present on the gills situated underneath the top of the mushroom. A cap measuring 10 cm in diameter has the potential to produce up to 100 million spores every hour.

2.7 Spoilage of bread

Flour and water are the two major products used for the preparation of bread. The dough is prepared from flour and g through fermentation for the desired microorganisms to grow at the required limit which causes spoiling and high growth of bacteria's that reduces the capacity of gas which is required for the dough to rise. Moldness and ropiness are the two types of bread spoilage

During the production process of bread, it can be baked at a relatively moderate temperature, therefore there are less Chance of the survival of microorganism. Contamination occur only when Cooling is done as well as during the packaging and handling from the environment.

2.8 Diseases of fungi in human

The presence of ropiness in bread is typically attributed to bacterial proliferation, which tends to be more common in homemade breads. The primary causative bacterium is Bacillus subtilis or B. licheniformis. Some bacteria have the ability to generate spores that can withstand high temperatures during baking. Under certain conditions, such as exposure to heat treatment, these spores have the ability to develop into vegetative cells. The hydrolysis of gluten, which is the protein found in bread flour, occurs through the action of proteinases, resulting in a ropiness texture. Amylases also catalyze the hydrolysis of starch, resulting in the formation of a viscous texture known as ropiness. Ropiness is characterized by the formation of a soft and adhesive surface that appears yellow to brown in hue. Additionally, it is accompanied by a scent.

Ropiness, a bacterial infection, is a significant cause of bread spoiling, second only to mold. It is more prevalent during the summer months, when the environmental conditions are conducive to bacterial growth. Bacillus subtilis is the primary cause of ropy bread, additionally, bacillus licheniformis, bacillus megaterium, and bacillus cereus have also been associated with this problem. The incorporation of rope into bread manufacture can result in spoiling, hence presenting a potential health risk. The abundance of bacillus subtilis and bacillus licheniformis in food can

lead to the growth of mold and the occurrence of foodborne disease. There is evidence from Ybar et al. (2012) and Rumeus and Turtoi (2013) that consuming stale bread can cause foodborne illness.

2.9 Spoilage factors

The spoiling of bread usually happens owing to the absorption of moisture content during storage leading to fungus development at excessive humidity and temperature.

Prior to the storage and packing procedures, the grains undergo decontamination to minimize the presence of microorganisms and extend their shelf life.

Physical - spoiling is cause by physical losses, which occur owing to the post processing contamination of packaging material.

Physiological – are caused to warming of grains, temperature, humidity and the oxygen level.

Biological – micro-organisms are the cause of biological losses. (Uobabylon, Edu. 19 Et Al., 2015).

Here is a summary of bread spoilage:

The green spored mold is known as penicillin expansum.

The organism responsible for bread mold is Rhizopus stolonifer.

The observed characteristics consist of a white, cotton-like mycelium and black dots.

Neurospora sitophila is a type of red bread mold.

The presence of Bacillus stibtilis (Bacillus mesentericus) causes the characteristic ropiness in homemade breads.

The formation of a viscous texture is caused by the breakdown of flour protein by the action of a proteinase enzyme produced by the bacillus, followed by the encapsulation of the bacillus.

2.10 Identification of fungi: Criteria for identification of yeast and mold

Fungi are identified by assessing their growth rate in combination with other factors.

Characteristic of colonial morphology

Observation at a microscopic level

The growth rate is a valuable observation to consider when studying fungus. Nevertheless, this information may have limited significance as the growth rate of specific fungus can vary depending on the inoculum. The average growth rate of dimorphic fungi, such as Blastomyces detatitides and Histoplasma capsulatum, spans a period of 1 to 4 weeks.

Zygomycetes colonies appear within a 24-hour timeframe.

Similarly, the saccharomyces yeast produces colonies that are evident during a period of 3-5 days.

Colonial morphology is a significant characteristic of fungi, although its usefulness in identifying mold is limited due to the natural variance among isolates and the fluctuation regarding the colonies, they were grown under various culture conditions. When examining the physical features of fungus colonies during the colonial growth stage, it is crucial to consider the specific types of nutrient-rich substances used for cultivation and the environmental circumstances in which the incubation takes place. Histoplasma capsulation is characterized by the presence of a white yeast mold on BHI agar. When cultivated on the same media with blood enrichment, it appears as yeast. Colonial morphology can serve as a complementary source of information to enhance the findings gained from microscopic investigation.

2.11 Yeast identification criteria:

Whether ascospores are produced or not

If they produce ascospores, please provide information on the look and quantity of ascospores.

Morphology of the vegetative cell

Form and dimensions

Asexual reproduction technique

Generation of mycelium, pseudomycelium, or lack of mycelium generation

Visible growth's pigmentation

2.12 Criteria for identification of mold

Hypae can be classified into two types: septate or non-septate.

Mycelium can have either a transparent or a black appearance.

The color of mycelium can either be pigmented or unpigmented.

Sexual spore's classification

Classification of Asexual spores

Features of aerial hyphae or spores:

Sporangia: characteristics of size, color, form, and position

The characteristics to consider are the conidia, their organization (whether in chains, budding, single, or in clusters resembling mosses), as well as the shape and arrangement of sterigmata or phiallides.

Organization of the soprangiophore or condiophore: The characteristics of interest are the type of branching (simple or branched), as well as the size and shape of the collumela at the tip of the sporangiophore.

A single condiophore or a cluster of conidiophores.

The presence of distinctive features such as theft, rhizoids, and apophysis is seen (Gaurab Karki, 2018).

2.13 Parameters for mold detection

Hyphae can be classified into two categories: septate and non-septate.

Mycelium can exhibit either a translucent or an ebony coloration.

Mycelium can display either pigmented or unpigmented colors.

Taxonomy of reproductive spores

Taxonomy of Asexual spores

Features of aerial hyphae or spores:

Sporangia: features encompassing dimensions, hue, structure, and positioning

The relevant factors to take into account are the conidia, their arrangement (whether in chains, budding, single, or in clusters resembling mosses), as well as the morphology and disposition of sterigmata or phiallides.

The organization of the soprangiophore or condiophore: The key attributes of concern are the branching pattern (either simple or branched) and the dimensions and configuration of the collumela located at the apex of the sporangiophore.

Either a solitary condiophore or a group of conidiophores.

Presence of unique anatomical features: pilfered, rhizoids, apophysis, etc. Gaurab Karki, 2018

2.14 Fungal contamination in bread control

Various methodologies can be employed to manage the proliferation of mold on bakery goods namely; reformulation, freezing and most commonly. The use of preservatives.

Reformulation

Refers to the process of reducing the amount of water present in a bakery product in order to increase its shelf life. Product reduction can be accomplished by dehydrating it using methods such as evaporation or freeze-drying, or by adding high osmotically active substances like sugars and salts straight into the food. The extent of a decrease is of practical importance in rendering a food non-perishable. The degree of sensitivity to a particular stimulus varies significantly among microorganisms in different environments (Gourama 2015). The water in solutions of sugars and salts becomes more concentrated with crystalloids. Furthermore, bacteria experience direct osmotic injury as a result of the high concentration of these compounds. The reason for this occurrence can be related to the diverse effects of limited water availability on all metabolic activities, as all cellular chemical reactions require a water-based environment. Ensuring an appropriate water activity (Aw) level is crucial for preventing the establishment of mold in bread products (Saranraj and Geetha 2012).

Freezing- Food preservation is a highly efficient technique for extending the shelf life of bakery items, particularly those that contain cream. Swift freezing is essential for controlling the formation

of ice crystals. Delayed freezing results in the creation of large ice crystals. According to Banwart (2013), these sizable crystals have the capacity to disrupt the integrity of membranes and the internal organization of cells.

Preservatives – Yeast is mostly used to control the growth of baked goods. The Code of Federal Regulations (CFR) defines a preservative as an antimicrobial agent used to protect food by preventing the growth of germs and the subsequent spoilage. Preservatives are classified into two categories. Consisting of both synthetic and organic chemicals. The authorized chemical mold inhibitors for bread comprise acetic, sorbic, and prolonic acids, together with their respective salts. The ingredient statement lists natural food preservatives, such as cultural product, by their precise names (Saranraj and Geetha 2012).

2.15 Economic losses associated with bread

Bread is susceptible to spoilage problem. The spoilage include microbial Spoilage with mold growth being one of the significance of bread and its impact on bakeries is of a major concern. penincillium spp, Rhizopus spp, and Aspergillus spp are the mold involved in the spoilage of bread. Due to mold spoilage, the economic losses of bread are between 3% and 6% depending on the methods of processing, season, and the types of product. The mycotoxins production by some mold are additional are additional economic loss associated with bread. The concentration of nutrients may determine the rate of nutrients.

2.16 Microscopic Observation

Microscopic features of fungus are typically precise and provide specific identification based on characteristic features, spore production mechanisms, and the shape and arrangement of spores. Nevertheless, the dimensions of hyphae are significant for the purpose of fungus identification. The preparation and execution of staining with lactophenol cotton blue was carried out for microscopic analysis.

2.17 Effect of Biopreservatives

Fungal species have the capacity to generate poisons and induce disease. Determining the harmful bacteria is essential for both diagnostic applications and environmental surveillance. Historically, mold has been the predominant source of microbial decay in bakery products. Fungal activity leads to a loss of approximately 2-10% of bread output. Mold infestation not only alters the taste and look of food, but also diminishes its overall quality by producing mycotoxins.

The application of microorganisms and their byproducts to inhibit spoilage and extend the duration of food storage, referred to as bio-preservatives, has gained significant attention due to increasing consumer expectations in recent times. Lactic acid bacteria (LAB) are fascinating microorganisms that possess great potential as bio-preservation agents. The introduction of a laboratory strain with antifungal properties into a starting culture led to a reduction in the required quantity of calcium propionate as a preservative (Hassan and Bullerman 2015).

2.18 Mycotoxins

Mycotoxins are tiny compounds that are generated as secondary metabolites by filamentous fungus. The metabolites in question form a diverse collection that varies in terms of toxicity and chemical composition. They are classified together solely because they have the ability to cause illness and mortality in humans and other vertebrates. Aflatoxins are a specific class of mycotoxins that are synthesized by the fungus Aspergillus flavus. Scientists became aware that mycotoxins could potentially be lethal, which led them to consider the existence of additional hidden mold byproducts that could also be harmful. Subsequently, the mycotoxin classification was expanded to encompass various fungal toxins that were already known, such as the Ergot alkaloids. Additionally, it included certain compounds that were initially identified as antibiotics, like patulin, as well as several newly discovered secondary metabolites found through specific screenings for mycotoxin detection, such as Ochratoxin A. According to Turner et al. (2014) and the FDA (2015), mycotoxins are the only harmful low-molecular-weight fungal metabolites, while substances like Ethanol are not classified as mycotoxins until they are present in significant concentrations.
Presently, there are about 400 mycotoxins that have been identified worldwide. Due to their high heat stability, these chemicals pose a possible health risk to both people and animals. The presence

of mycotoxins in contaminated products leads to a decline in their marketable quality, resulting in significant economic losses (Zinedine Et.al., 2015).

2.19 Formation of Bread making

Internationally, recipes used by professional bakers employ a system of measurement known as baker's percentages. The flour is often measured at 100% weight, whereas the other ingredients are stated as a proportion of that weight. Weight-based measurement is superior to volume-based measurement in terms of accuracy and consistency, especially when dealing with substances that are difficult to measure precisely. The water-to-flour ratio is the critical factor in a bread recipe, exerting the most influence on texture and crumb structure.

Hard OS Wheat flours have an absorption rate of around 54%. When used to make bread dough, these flours produce a loaf that is light and has a fine texture. The majority of artisan bread recipes typically include a water content ranging from 60 to 75%. Within the context of yeast bread. Increasing the water content leads to a greater amount of CO2 bubbles, resulting in a rougher texture of bread. Using one pound (450g) of flour will produce either a regular loaf of bread or two French loaves. Commercial bakeries often incorporate calcium propionate to inhibit the proliferation of molds (Seiler, 2013).

2.20 Bread production procedure

Globally, professional bakers utilize a measurement technique called baker's percentages. The flour is often quantified at a 100% proportion, whereas the other ingredients are expressed as a weight percentage in relation to the flour. Weight-based measurement is a more accurate and dependable approach in comparison to volume-based measurement, particularly when dealing with compounds that pose challenges in measurement. The water-to-flour ratio is the pivotal element in a bread recipe, since it exerts the most influence on the texture and structure of the bread.

The absorption rate of Hard OS Wheat flours is approximately 54%. When utilized in the production of bread dough, these flours yield a loaf with a refined texture and airy consistency. Artisan bread recipes often have a water content that falls within the range of 60 to 75%. In the realm of yeast bread. The augmentation of water content induces the generation of a greater number of CO2 bubbles, hence yielding a more granular consistency in the bread. Utilizing a single pound

(450g) of flour will yield either a standard loaf of bread or two French loaves. Calcium propionate is frequently used in commercial bakeries to prevent the growth of molds (Seiler, 2013).

2.21 Importance of identifying mold contamination in bread.

Fungus species are toxigenic and pathogenic. So identifying the unfavorable microbes is important for the diagnosis and the environment monitoring. Historically, moldiness has been the main type of microbial spoilage of bakery products. About 2-10% of bread production are not done properly due to the fungus activity. Mold contamination may not only determine the changes but also taste, color but also loss in the quality of food because of the formation of mycotoxins.

Contamination of bread with molds may occur in the following steps:

Cooling, storage, transport, cutting and packaging.

2.22 Locally made bread in Liberia

Fantain bread ingredients

Water, flour, yeast, baking, powder/soda

3 cup flour, 2 teaspoon of butter, 3 eggs, sugar as need, warm milk, baking powder, 1 teaspoon of yeast.

Preparation of Fantain bread

Add all the ingredients together and make a dough. Mix it with your hands and it compiles together as a complete dough with the addition of one cup of warm water. Cover it to allow yeast growth within 1 hour and after that you allow it to rise for 45min and bake for at least 15.20 Min.

Package and storage of Fantain Bread

After baking, the fantain bread is taken to a storage for cooling and packaging for about half an hour. After that, the bread is ready for delivery at various vendors and most especially Fula shops for human consumption.

Lebanese bread – a new type of bakery bread in a round form. It is another soft texture bread made by Liberian for consumption

Lebanese bread ingredients

Water, flour, yeast, baking, powder/soda

1 ½ cups refined flour (whole white flour), ¼ cup atta, 1 teaspoon dry yeast, ½ teaspoon sugar. 2-3 teaspoons of caramel, 1 teaspoon oil.

Preparation of Lebanese bread

Over temperature: 400 f-240 C. leave in a draught free place to froth. Mix the yeast together with the flour, add it to the flour mixture along with salt and a bit of water. After mixing by hand and completely compiling the ingredients, spread it in an Aluminum plate and place it in the oven for 15-25 minutes for baking.

Package and Storage for Lebanese Bread

After baking, Lebanese bread is separated from each other s and placed at room temperature for storage until delivery time.

Shortbread – is a traditional bread usually made from white sugar, butter, and plain wheat flour.

Shortbread ingredients

Flour, sugar, salt, baking powder, oil/butter

Preparation of Shortbread

Preheat the oven at 16oc/140c.

Combine the flour with the butter and sugar until it becomes a pale and silky consistency. Gently combine the flour until it forms a cohesive mixture. Exert manual pressure on the mixture to compress it into a cohesive mass. Effortlessly flatten the dough and carve it into desired forms,

then position it in the baking pan and transfer it to the oven for around 15 minutes to allow it to settle.

Package and storage

After baking shortbread is slice and store within a white bread container to be sold by vendors for human consumption

Rice bread – is a locally made bread from rice flour rather than white flour. Being free of gluten.

Rice bread ingredients

Rice flour, baking soda, salt, banana, and oil

A cup of white flour, a cup of brown rice flour

A teaspoons of xanthan gum, a teaspoon unflavored gelatin

A tablespoon sugar, a teaspoon salt. 2 tablespoon egg, 2 cups of milk powder

A teaspoon dry yeast, 1 egg, vinegar.

A tablespoon oil and 2 cups water

Preparation of Rice bread

In order to produce a high-quality rice bread, it is essential to ensure that all the ingredients are stored in a hygienic environment prior to the baking process. Combine the rice flour and salt. The flours must be thoroughly If the mixture is excessively dry, incorporate sugar, milk powder, eggs, vinegar, oil, and water into the dough. Place it in the bread pan and let it ferment in a moist setting. The dough is placed in an oven and cooked at a temperature of 175 degrees Celsius until the crust is thoroughly baked. Allow it to cool for a duration of ten minutes before proceeding to cut it.

Package and storage of Rice bread

After baking, rice bread is slice and store within a white bread container and can be sold by vendors or bakeries owners for consumption.

Fula bread – as known in Liberia is a locally made bread with high texture usually made by the Fula tribe.

Fula bread ingredients

Water, flour, yeast, baking, powder/soda

Water, flour, yeast, baking, powder/soda

3 cup flour, 2 teaspoon of butter, 3 eggs, sugar as need, 2 teaspoon of yeast, 2 teaspoon of baking powder, warm milk

Preparation of Fula bread

Add all the ingredients together and make a dough. Mix it with your hands and it compiles together as a complete dough with the addition of one cup of warm water. Cover it to allow yeast growth within 1 hour and after that you allow it to rise for 45min and bake for at least 15.20 Min.

Package and storage of Fula Bread

After baking, the Fula bread is taken to a storage for cooling and packaging for about half an hour. After that, the bread is ready for delivery at various vendors and most especially Fula shops for human consumption. **Fula bread** – as known in Liberia is a locally made bread with much hard texture and it is dominantly made by the Fula tribe and if baked well these breads become tasty and yummy for human consumption.

CHPATER THREE

3.0 Overview of chapter three

This chapter looks at the research design, methods and materials, target populations/sampling methods, methods of collecting data, methods of analyzing data and ethical consideration of the research.

3.1 Research Design

The most important scientific approach to research is an experimental research design, Mostly relating to laboratory test, procedural experimental research design involves collecting a quantifiable data and performing analysis on the research. Therefore, the primary source of original data collection process of original evidence which is the understanding of the fungi spoilage of bread in Monrovia, Liberia

3.2 Research Setting

Samples of breads was taken from a targeted local bakery within Monrovia, Liberia.

3.3 Target Population / Sampling Method

To this study, the targeted bakeries owner was 4 (four) that I visited for the samples of bread during this study from November to December 2023.

3.4 Materials and Methods

Breads samples
Sterile plastic bags / sterile polyethylene bags
Sterile scalpels
Sabouraud dextrose broth

3.5 Sample collection and processing

Bread samples was purchase from a targeted local bakery in Monrovia, Liberia. The samples are Fula bread, Fantain bread, Lebanese bread, Rice bread, Short bread. Samples were collected from the bakeries into a sample container (sterile plastic bag) and was relocated to the laboratory for a week so that spoilage to occur.

Fungal isolate

The samples were diluted, and 0.2ml of the diluted solution was used to inoculate the sabouraud dextrose agar (SDA) acomodating 1% chloramphenicol, which was used to prevent bacterial growth. The plates that were treated with a little amount of a substance to prevent disease were placed in a controlled environment at the normal temperature of the room for a period of 72 hours. During this time, the colonies on the plates expanded and were tallied. The process of transferring the colonies to new plates was then repeated, and the new plates were housed in slanted containers containing a specific type of growth medium called SDA. This was done in order to further study and identify the colonies.

Characterization and identification of isolates:

The isolated colonies was identify both macroscopically and microscopically. The color, size, and texture of the colony were evaluated, and a microscopic investigation was conducted using a stain. One droplet of the isolate was deposited onto a glass slide. A small amount of the fungal isolate was placed on clean glass slide and covered with the cover slip to prevent the formation of bubbles. The slide was view under the microscope and the fungal were identified.

Measurement of the bread samples' moisture content

Initially, the weight of the crucible was measured. Subsequently, a 10g piece of bread was added to the crucible and its weight was measured again. The crucible and its contents were subsequently dehydrated in an oven at a temperature of 105 degrees Celsius for a duration of 3 hours. The weight was determined after the substance had cooled. The moisture content percentage was determined using a mathematical technique.

The Initial weight of samples plus the final weight of bread times 100

Measurement of the pH levels in the bread samples

A mixture was prepared by combining one gram of bread samples with 10ml of deionized water. The mixture was then tested using a calibrated meter of the PH, which was equipped using pH buffers of 4.0 and 7.0. The pH values of the mixture were recorded.

3.6 Method of samples collection

To this study, samples collection was done from a targeted local bakeries in Monrovia, Liberia and was taken to microbiological laboratory for analysis and ethical consideration was observed.

3.7 Method of data Analysis

The data was evaluated using tables with frequency distribution and percentages.

3.8 Ethical Consideration

The procedure of collecting samples for this study involves the following: a letter was obtained from my supervisor Prof. Dr. H. Kaya Suer and it was submitted to the management of the clinical laboratory asking for their approval to conduct this laboratory procedure at their facility. The bakeries owners' right and information was fully protected.

CHAPTER FOUR

4.0 Overview of this chapter

This Chapter Comprises of data presentation through tables and figures, findings and discussion of findings.

Table one.

Samples	Fungus Counts
Fula Bread	3.0×10^3
Fanti Bread	1.2×10^3
Short Bread	2.1×10^3
Lebanese bread	4.3×10^3
Rice Bread	2.2×10^3

Fungal counts of the samples

Isolate	Macroscopic properties	Microscopic Characteristic	fungi
1	White color colonies at first and black later. pale yellow reverse slide	Septate hyphae Conidiosphores bearing with thick-walled conidia were seen	Aspergillus ssp
2	dark brown colonies with the reverse side was grey	non-septate hyphae, with tea simple branchal bearing spoiangiophores	Rhizopus ssp
3	Colonies that are green and have a silky texture The obverse side exhibited a pale yellow.	The hyphae are septate and have smooth walls. The conidiosphores have chains of conidia.	Penincilliom ssp
4	Colonies with a dark olive color. The opposite surface exhibited a black.	The hyphae are septate, having conidiosphores that are straight and carry conidia.	Cladosporium ssp
5	Grey colonies	The sporangia are black and contain zygospores. The sporangiosphores were elongated and the hyphae were non-septate and branching.	Mucor spp

Table 2.

Characteristics at both the microscopic and macroscopic levels of the fungus isolated from the samples.

Table 3.

Frequency of Fungi presence in commercial bread samples

Bread samples	Aspergillus Spp	Rhizopus spp	Penincillum spp	Clodsoporium spp	Mucor spp
Fula bread	+	+	+	+	+
Fanti bread	+	+	_	_	+
Lebanese bread	+	+	_	_	+
Short bread	+	_	+	_	_
Rice bread	+	+	+	+	+

Table 4.

Moisture content % and pH of bread samples

	samples	Moisture content expressed as a percentage	PH
	Fula bread	44.5	5.94
	Fantain bread	44.2	5.98
	Lebanese bread	44.7	5.90
	Short bread	44.3	5.96
	Rice bread	44.4	5.95

4.1 Results

The fungal counts of the samples varied from 1.2x103 cfu/ml to 4.3x103 cfu/ml. Specifically, Fula bread had a fungal count of 3.0x103 cfu/ml, Fanti bread had a fungal count of 1.2x103 cfu/ml, Lebanese bread had a fungal count of 4.3x103 cfu/ml, Short bread had a fungal count of 2.1x103 cfu/ml, and Rice bread had a fungal count of 2.2x103 cfu/ml. The fungal species isolated from the bread samples were identified and classified based on their macroscopic and microscopic properties. These species include Aspergillus spp, Rhizopus spp, Mucor spp,

Penicillum spp, and Cladosporium spp. It was observed that Aspergillus spp was present in all of the bread samples, while Rhizopus spp and Mucor spp were present in all samples except for the Rice bread. Penicillium spp was present in all bread types except Fanti bread and Lebanese bread, but Cladosporium spp was found in both Fula and Rice bread. The moisture level varied from 44.2% to 44.7%, while the pH varied from 5.90 to 5.98 consecutively. The Fanti bread had a moisture content of 44.2% and a pH value of 5.98. Similarly, the moisture content of Lebanese bread was measured to be 44.7%, while its pH value was determined to be 5.90. The shortbread sample had a moisture content percentage of 44.3 and a pH value of 5.96. The rice bread had a moisture content % ranging from 5.90 to 5.98 consecutively.

4.2 Discussion

The fungal counts of the commercial bread samples varied within a certain range. 1.2x103 cf/ml to 4.3 x103 cfu/ml. The fungal count in Lebanese bread was the highest, measuring 4.3 x 103 cfu/ml, whereas Fanti bread had the lowest value of 1.2 x 103 cfu/ml. The elevated fungal counts were likely a result of inadequate previous baking and handling of bakery products, as the bread making operations often eliminate fungal spores. Potential contamination of bread can arise from airborne particles, baking surfaces, equipment, and individuals involved in the chilling, wrapping, or slicing processes. Moreover, the presence of moisture condensation on the bread's surface can be attributed to the packing, which creates favorable conditions for the formation of mold. The species that have been identified are Aspergillus spp, Rhizopus spp, Penicillium spp, Cladosporium spp, and Mucor spp. The identified isolates, Aspergillus SPP and Penicillium SPP, are classified as fungi that are responsible for bread deterioration. The consistent presence of fungi in the bread samples indicated that Aspergillus spp was detected in every samples, while cladosporium spp had the lowest occurrence rate and was isolated from Fula and Rice bread samples. The capability of creating Amylolytic andpropronate socymes from the isolated fungus could be a substantial factor to the spoilage of bread.

Utilizing acidic chemical preservatives, such as propionates, palpitates, or sorbets, contributes to the low pH levels observed in the bread samples. The presence of elevated moisture levels and a low pH in bread creates a conducive environment for the growth and proliferation of fungi, leading to spoiling. The decay of bread caused by fungi is influenced by its chemical makeup, pH, and moisture level. The proliferation of mold has been the primary determinant of the bread's longevity.

The occurrence of these fungal contaminations in a considerable quantity of bread poses a risk to public health, as certain types of fungi are capable of producing mycotoxins that are harmful to humans. Therefore, it is essential to maintain adequate hygiene during all stages of bread production in order to inhibit the growth of these fungi. Consuming spoiled bread is not advisable, and it is highly suggested to use a safe food-grade chemical preservative throughout the bread making process.

CHAPTER FIVE

5.0 Summary of this chapter

This chapter of my research article examines the ramifications of the findings, the strength of the investigation, and the constraints of the study**.**

Implication of the findings

A study on the fungal organisms responsible for bread spoilage in Monrovia, Liberia was conducted November to December 2023 is an in vitro study. Findings from the study can be used to help raise awareness in order to reduce the high intake of spoilage bread among community dwellers in Liberia. The findings can also lay the foundation for public health practitioners in helping to educate community dweller of some diseases caused by high intake of spoilage bread. The findings shows that Penicillium spp, Asporgillus spp, Cladosporium spp are the most common molds found in bread spoilage. Finally, the results indicate that The Lebanese bread had the greatest fungal counts, measuring 4.3 x 103 cfu/ml, while Fanti bread has the most minimal fungal counts, measuring 1.2 x 103 cfu/ml. The pH values ranged from 5.90 to 5.98 for both types of bread, respectively.

5.3 Strength of the study

A study on the fungal organisms responsible for bread spoilage in Monrovia, Liberia is a laboratory base study so therefore all the findings are scientifically proven and accurate. This study also covers vast majority of the high intake of spoilage bread therefore it finding can be used generally to recommend solution in curtailing or reducing the intake of spoilage bread. This study is the first of its kind to be conducted in Liberia therefore, a lot of discoveries were made. The study discovered the fungi such as: The molds commonly detected in spoiled bread include the identified mold species include Penicillium spp, Mucor spp, Rhizopus spp, Aspergillus spp, Cladosporium spp, and other commonly seen molds.

CHAPTER SIX

Conclusion and recommendations

This chapter look into the climaxing of the research work and It also provides recommendations based on findings as well as recommendations for future investigations.

6.1 Conclusion

In summary Bread can become spoiled by fungi. The information gleaned from the study will assist in informing consumers about the potential health risks associated with the organism's growth and the toxins found in spoiled bread, as well as potential remedies. The only way to reduce bread decomposition after baking is to employ safe food grade and chemical preservatives, as fungus deterioration is an expensive issue for bakeries.

6.2 Recommendations according to the research findings

Based on the results the researcher suggest the following. The bakeries should prioritize the implementation of food safety management in other to minimize the spoilage of bread. Moreover, there should be door to door campaign carry out by the government to create awareness of it citizens in order to curtail the danger of consuming spoilt bread and the fungal diseases associated with it. Lastly, in order to stop the sales of spoilt bread on the market the health authorities should carry out a day to day inspection of bakeries and as a citizen I am urging my people to always eat freshly baked bread and not to eat any bread with mold growth, physical changes or have lasted over a day

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