BAKED GOODS FUNGI SPOILAGE AND ANTI-FUNGI ACTIVITIES

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Abstract
Bread products are an important part of a balanced diet. It is an excellent source of nutrients, such as macronutrients and micronutrients essential for human health. Baked goods are prone to fungal decay. Many filamentous fungi such as Rhizopus, Mucor, Aspergillus and Fusarium are implicated in bread spoilage due to improper handling and sanitation. This study investigated spoilage of moldy baked goods and antifungal activities. The 30 bread samples used for this study were collected from various vendors in Enugu and exposed for 7 days at various locations around the University and observed daily for spoilage. Glassware used for this study was properly sterilized in a hot air oven at 160^0C for two hours, other materials were autoclaved at 121^0C for 15 minutes. The culture medium used for this experiment was Sabouraud Dextrose Agar (SDA), which is known to support the growth of fungal organisms only. Organisms associated with bread spoilage are strictly fungal organisms including; Rhizopus spp, Aspergillus spp, Mucor spp, Penicillium spp and Fusarium spp. These isolates are identified bacteriologically by their cultural morphological characteristics. After examining the samples, it was discovered that Rhizopus is the fungus commonly found in bread. Spoilage of baked foods can be a food health hazard and can cause a mild form of foodborne illness. Chemicals are harmful to human health, so the use of natural agents helps maintain human health. Does not affect the taste of food. Clove, lemongrass, cardamom, citrus and edible oil are commonly used in food products. These products also act as control measures for these fungi. This study focuses on fungal spoilage of bakery products and its control measures.
Keywords: *fungi, bread, anti-fungi, spoilage*

Introduction

Baked goods are basically staple foods in most countries and cultures. The most common products are bread, buns, cupcakes, cookies, pizza base, toast, etc. Grains used in baked goods are an important source of nutrients in our diets, providing us with most of the calories in our diet and nearly half of our protein. terms. The nutrients in baked goods are carbohydrates, proteins, fats, vitamins, calcium, iron, minerals, starch and energy. Baked goods have been with us for thousands of years. Bakers began making bread at home in an oven, using mills to grind grain into flour for their loaves. The spectacular appeal of baked goods encouraged cooking across Europe and spread to East Asia (Hunt and Robbins, 2009). Baked foods that were once considered sick foods have become essential foods for the majority of the population. The importance of baked goods has increased, especially the use of natural grains and other natural ingredients. The bread industry in India is the largest of the food industries, with an annual turnover of around 3 billion rupees (Guynot, Marin, Sanchis and Ramos, 2005). India is the second largest producer of cookies. The main bakery products are breads and cookies account for more than 80% of the total bakery products produced in the country. Commercial breads and crackers contain approximately 7.5% to 7.8% protein, respectively (Bartkiene, Juobeikiene, & Vidmantiene, 2008).

Bread products are good targets for fiber enrichment. The fiber enrichment of some bakery products using a substance containing a percentage of short-chain fructo-oligosaccharides has been tested recently (Vagelas, Gougoulias, Nedesca and Liviu, 2011). Baking ingredients are wheat flour, water, sugar, salt, eggs, shortening, yeast, baking soda, baking powder, cornstarch, milk, butter or margarine, honey, yogurt, essence, cocoa powder, chocolate bars, fruit jams, sweeteners, etc. . . Broken bread products can be defined as bread products that are broken or spoiled until they become undesirable for human consumption. Baked goods are prone to spoilage issues. These include physical, chemical and microbial degradation. Consequently, its important economic and commercial value has been lost due to poor management. Economic losses associated with bread products are the possibility of mycotoxin production. Mold spores are usually killed when baking fresh bread and other baked goods. Therefore, a moldy bread product must be contaminated by air, bread surfaces, utensils, food handlers or raw ingredients, or after baking during refrigeration, slicing or cleaning. This means that any spoilage issues caused by mold should occur after cooking. Fungal spores are higher in the summer months than in the winter months due to air pollution in hot weather and more humid storage conditions. Fungal decomposition has caused unwanted odors and is often visible on the surface of the product. The most

common fungi found in bread as one of the bread products are Rhizopus, Aspergillus, Mucor, Fusarium sp.

The scientific names of the fungi that grow on bread are; Rhizopus nigricans and Mucor stolonifer (Banwart, 2004). There is little difference between the two and they are both commonly known as "bread tray". They are the first to "come" and become a piece of bread. Subsequently, many others may follow, such as Aspergillus and Penicillium (Hocky, 2008). Yeast is used in bread dough to release CO2 and make bread fluffy and chewy. Bread is one of the oldest prepared foods. Evidence from 30,000 years ago in Europe has revealed starch residues in rocks used to thresh plants. It is possible that during this period the starchy extract from the roots of plants, such as cattail and ferns, was spread on a flat stone, put on a fire and baked into a primitive flatbread form. . Around 10,000 BC. J.-C., with the arrival of the Neolithic and the expansion of agriculture, cereals became the basis for making bread. Yeast spores are everywhere, even on the surface of cereal grains, so any lumps left to sit will grow naturally. There are several sources of sourdough available for early bread. Airborne yeasts can be used by leaving the unbaked dough exposed to air for several hours before baking. Pliny the Elder reported that the Gauls and Iberians used skimmed beer foam, used a paste made from grape juice and flour that began to ferment, or wheat bran fortified with wine, as a source of yeast. . . The most common source of sourdough is the aging of a

piece of dough from the day before it is used as a form of sourdough (Seiler, 2000). In 1961, the Chorleywood bread process was developed, which used intense mechanical working of the dough to dramatically reduce fermentation time and the time needed to make bread. The process, in which high-energy mixing allows the use of low-protein grains, is now widely used around the world in large flour mills. As a result, bread can be made very quickly at low cost to both the manufacturer and the consumer.

The incidence of bread spoilage caused by these fungi has increased in recent years, probably because more bread is being made without preservatives and raw materials such as bran and seeds are often added. Spoilage in these baked goods can be a food health hazard and can cause a mild form of foodborne illness. The consumption of these products is associated with foodborne illnesses. Bread products are usually wrapped in plastic film after cooking and cooling and consumed within 1-2 months. Post-process contamination is not available. Contamination of fungal organisms by these types of products usually occurs during the cooling period after cooking, as the cooking temperature is usually sufficient to remove previous contamination. This study will focus on fungal spoilage of baked goods and its control measures.

Materials and Methods

The 30 buns used for this study were purchased from various vendors in Eldoret, Kenya. The samples collected were brought in a sterile polyethylene bag to the laboratory for analysis. The culture medium used for this experiment was sabouraud dextrose agar (SDA), which is known to support the growth of fungal organisms only. The medium was prepared according to the manufacturer's instructions. All glassware used for this study was properly sterilized in a hot air oven at 160 ° C for one hour. The other materials were autoclaved at 121 ° C for 15 min. The methods used in these experiments were performed according to the criteria recommended by the following researchers (Alexander (1999), Harrigan (1988), Dubey and Maheshawi (2004) .8.5 g of salt was weighed with a balanced triple beam. for 100 ml of water The two mixtures were mixed and sealed with aluminum foil and autoclaved at 1210 ° C for 15 minutes Three test tubes containing 9 ml of sterile normal saline were in a rack 1 gram of each sample was dissolved in the first test tubes and mixed thoroughly.0.1 ml of the sample was aseptically piped into the first test tube and mixed, this was repeated until the last tube (10-3) .Then 1ml of the last was discarded tube.The table was wiped with 70% ethanol using cotton., the purchased samples were labeled and placed on the table accordingly.The samples were placed on the table with a bunse burner n in lit to keep the area sterile of work and no unwanted organism. A total of 30 buns. Samples were used in petri dishes, allowed to gel.

Plates were blotted upside down, sealed with paper tape and then incubated at 37°C for 1 week for colony formation. The number was determined by counting the corresponding colonies observed after 1 ml of serially diluted samples. Placement techniques were used for discrete colonies in 1 ml inocula. The number was recorded in colony forming units per ml (CFU ml). A small portion of each undercultured colony was cut using a sterile dissecting blade. It was then removed with sterile forceps and placed on a new sterile glass slide; The slide is covered with a coverslip, which is hidden at an angle to the new petri dishes. Petri dishes were left in the bank for 5 days. The coverslips were each carefully removed with forceps and placed on slides containing lactophenol. The slide preparation was carefully covered with coverslips except for air bubbles. Blotting paper was used to remove excess stain from the edge of the coverslip. Slides of each colony were prepared and viewed under object lenses at low (10X) and high (40X) magnification under a compound microscope. spore type, surface texture, plaque pigmentation and reverse pigmentation; colonies formed were also recorded. If there are no signs of mold growth, the box is marked with an "X". If mold growth is detected, the box is marked "Y".

Antifungal activity against fungal strain

Natural preventive control measures were used against fungal strains. Five controlled antifungals are used, namely cardamom powder, Syzygium aromaticum

powder, citrus juice, lemongrass juice and cooking oil. These controls were tested for antifungal activity against fungal strains of Mucor, Rhizopus, Aspergillus and Fusarium. These control measures are not harmful to humans and are easily digested in food. To prepare the solvent for the antifungal activity against fungal strains, 100 g of cardamom and Syzygium aromaticum were taken from a powder mill. 1 g of the powder was dissolved in 10 ml of distilled water and filtered through filter paper after 1.30 hours. The collected filtrate was sampled to determine its antifungal activity against the pathogen.

Clove powder : The traditional use of cloves is used as a food preservative and does not affect the taste of food. Clove is made up of many chemical compounds such as phenolic compound, phenolic acid, gallic acid and eugenol. These are the main bioactive compounds in clove. Clove powder is said to have strong antifungal effects against many strains. The main ingredient responsible for its antifungal activity is clove eugenol. Clove powder prevents fungal growth. Lemongrass Extract: Lemongrass is widely used in traditional medicine in many countries around the world. Lemongrass juice consists of many chemical compounds, it is alpha pinene, cissabinene hydrate, 1-8-cineole, geranium acetate, geraniol, terpinolene, b-caryophyllene, linalool, limonene, 3-myrcene, neral, geranial. The mixture of neral and geranial is called citral. These major citral fractions showed the highest antifungal activity. Lemongrass juice has been shown to be a powerful

fungicide and inhibitor of fungal growth. Leaf juices have an effect on fungi, such as reduced condition, loss of pigment, and alteration of conidiophore structure. It is effective in preventing fungal viability and spore germination.

Cardamom powder: The traditional use of cardamom as a food flavoring began in ancient times. Cardamom consists of many chemical compounds like cineole and other aromatic compounds like trypinyl acetate, terpineol, spathulenel, borneol, terpinene, etc. Other ingredients found in cardamom seeds include oil, starch, and protein. Cardamom contains 2% to 8% essential oil. The active ingredient in essential oil is cineole. Cardamom powder has been shown to have potent antifungal activity against fungi. It is effective in preventing the full growth of fungus.

Citrus Extract: Citrus lime flavors are used in beverages, candies, cookies, and desserts. Citrus juice contains many chemical compounds such as geranial, nerol, geranyl acetate, geraniol, beta-caryophyllin, nerol, neryl acetate. The main ingredient in citrus fruits is limonene. Citrus juice has strong antifungal activity against many fungal strains. Strongly inhibits sporulation and growth of fungal pathogens.

Cooking oil: Soybean oil, rich in oil seeds, is very useful for cooking and also works as an antifungal. In particular, soybean oil is a key ingredient in nutritious foods. Soybean oil is often used as an additive to agricultural sprays to distribute

active ingredients. Soy products are not only a staple food all over the world, but are also used in industry and agriculture. This oil consists of 12% saturated acid and 80% unsaturated acid. Cooking oil has been reported to have strong antifungal effects.

Computation: This is done using the formula dc - dt / dc Where, dc - Control format type, dt - Target format type. Antifungal activity is tested by the arrow method in agar wells. Five control measures were tested against fungal strains. To do this, 20 ml of Astana and Hawker medium is poured into sterile petri dishes and allowed to settle. The tested mushroom colonies are exposed in four-dimensional Petri dishes. Wells with a diameter of 1 cm were drilled in all four corners using screwed screws. 20 ml of cardamom, Syzygium aromaticum, citrus juice, lemon juice and edible oil are poured into these wells. Dishes are heated at 270C for 48-72 hours. After movement, the formation of an exposed protective area is observed around the well of the dishes, indicating the presence of antifungal activity. The ban area is recorded.

Results and Discussion

Table: 1 Cultural, morphological characteristics and identification

Number	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
1	X	Little growth	Y	Y	Y	Y	Y
2	X	X	Y	Y	Y	Y	Y
3	X	X	Little growth	Y	Y	Y	Y
4	X	Little growth	Y	Y	Y	Y	Y
5	X	Little growth	Y	Y	Y	Y	Y
6	X	X	Little growth	Y	Y	Y	Y
7	X	Little growth	Y	Y	Y	Y	Y
8	X	X	Little growth	Y	Y	Y	Y
9	X	X	Y	Y	Y	Y	Y
10	X	X	Y	Y	Y	Y	Y
11	X	Little growth	Little growth	Y	Y	Y	Y
12	X	Little growth	Little growth	Y	Y	Y	Y
13	X	X	Little growth	Y	Y	Y	Y
14	X	X	Y	Y	Y	Y	Y
15	X	X	Little growth	Y	Y	Y	Y
16	X	X	Y	Y	Y	Y	Y
17	X	X	Little growth	Y	Y	Y	Y
18	X	X	Y	Y	Y	Y	Y
19	X	X	Little growth	Y	Y	Y	Y
20	X	X	Y	Y	Y	Y	Y
21	X	X	Little growth	Y	Y	Y	Y
22	X	X	Y	Y	Y	Y	Y
23	X	X	Little growth	Y	Y	Y	Y
24	X	X	Little growth	Y	Y	Y	Y
25	X	X	Little growth	Y	Y	Y	Y
26	X	Little growth	Y	Y	Y	Y	Y
27	X	X	Little growth	Y	Y	Y	Y
28	X	X	Little growth	Y	Y	Y	Y
29	X	X	Y	Y	Y	Y	Y
30	X	X	Y	Y	Y	Y	Y

Table.2 Bread observation at different handling environment.

Environment	Day 1	Day 2	Day 3	Day 4	Day 5
Wet bread in dark area	X	Y	Y	Y	Y
Bread in room temperature	X	X	X	Y	Y
Bread kept under the sun	X	X	X	X	X
Bread in airtight bag	X	X	X	X	X
Bread in refrigerator	X	X	X	X	X

Table 3 Cultural morphological characteristics and identification

ISOLATE	CULTURAL CHARACTERISTICS	MORPHOLOGICAL CHARACTERISTICS
Rhizopus spp	Large fluffy white milky colonies which later turns black as culture ages	Non-septate hyphal with uptight sporangiophore connected by stolon and Rhizopus, dark pear shaped sporangium on hemispherical columella.
Mucor spp	Cream white/large fluffy white colonies almost covering the whole surface	Sporangium comes out directly from the hyphal without stolon or rhizoids columella.
Penicillin spp	Large fluffy white colonies almost covering the whole surface	Non-septate branched hyphal enlarged at the apex to form conidophjorex they producer brownish black Ceridian in chains
Fusarium spp	Rapidly growing wooly to colt only lemon and yellow	Multicellular distinctive sickle shaped macro conidia.
Aspergillus spp	Very common colors of colony (black and white)	Conidia borne in 360 arrangements covering the upper 2/3 of the conidiophores

Table 4 Frequency of visible colonies

Isolate	(x) frequency/number of occurrence from both samples	5 frequency
Fusarium spp	2	6.06
Penicillium spp	3	9.09
Aspergillus spp	5	15.15
Mucor spp	11	33.33
Rhizopus spp	12	36.36

Where: Y = 33

Baked goods are spoiled due to fungal infection in the market. affecting product quality. After the incubation period, the average mushroom size of the bread sample is shown during storage for 7 days in Table 1. It has a mushroom size of 6-8 x 10 3 cfu. No fungal rate during the first two days of the study for thirty (30) samples was used. By the third day of the study, eighteen (18) of the 30 samples had low levels of fungi. However, all samples showed good fungal growth for four

to seven days. Muscle load increases as storage time increases. Therefore, the seventh day had the highest number of fungus cases in all experiments. Things that; Mucor spp, Fusarium spp, Aspergillus spp, Rhizopus spp and Penicillium spp. Table 3 shows the characteristics of differences based on cultural characteristics, colony morphology (cell size, shape, pigmentation and location). The results of this test show the area as an independent place where breadcrumbs are stored. Although the change depends on the taste of the bread. The variables (control variables) are ambient temperature, bread age and bread control.

Antifungal activity against fungal strain

Table 5: Antifungal activity against Rhizopus sp. by various solvent Control (mm) - 50.75.

S/N	Solvent Name	Inhibition zone (mm)
1.	Lemon grass juice	42.25
2.	Citirus juice	36.55
3.	Cardamom powder	37.25
4.	Edible oil	40.55
5.	Clove powder	38.75

In the case of Rhizopus, all five solvents show their growth inhibition. Citrus juice, powder and cardamom powder were found to be the most effective while vegetable oil and lemongrass juice were not effective against Rhizopus growth.

Table 6: Antifungal activity against Mucor Sp. by various solvent Control (mm) – 35

S/N	Solvent Name	Inhibition zone (mm)
1.	Lemon grass juice	23.25
2.	Citirus juice	9.75
3.	Cardamom powder	9.55
4.	Edible oil	23.75
5.	Clove powder	5.75

In case of Mucor, the solvent of clove powder, Cardamom powder & Citrus juice show the most active antifungal effect while the solvent of Lemongrass juice and Edible oil show to be the less effective against the Mucor.

Table 7: Antifungal activity against Aspergillus Sp. by various solvent Control (mm) – 48

S/N	Solvent Name	Inhibition zone (mm)
1.	Lemon grass juice	37.25
2.	Citirus juice	38.75
3.	Cardamom powder	34.55
4.	Edible oil	24.75
5.	Clove powder	40.75

The solvent of Edible oil, Cardamom powder and Lemongrass juice has been observed most effective while Citrus juice and Clove powder show that less effective against the Aspergillus.

Table 8: Antifungal activity against Fusarium Sp. by various solvent Control (mm) - 43.25.

S/N	Solvent Name	Inhibition zone (mm)
1.	Lemon grass juice	32.25
2.	Citirus juice	25.75
3.	Cardamom powder	29.55
4.	Edible oil	17.75
5.	Clove powder	9.75

In case of Fusarium all the five solvent has been observed active in the growth of inhibition against the Fusarium.

Fungal spoilage is a major cause of significant economic loss in the bread products obtained and can be planned as a source of mycotoxins related to general health problems. Therefore, the control of the sale of mushrooms and bread products is so important that the harmful effects can be reduced by general means. Antifungal activity against four fungal infections through five liquids such as Cardamompowder, Clove powder, Citrus juice, Lemon grass juice and clove powder shows positive effects on fungi as well as edible oil and Lemongrass juice

shows positive effect when citrus drinks are available. used as a detrimental effect on fungi and Cardamom powder shows adverse effects on the growth of fungi.

CONCLUSION

However, fungi can damage many types of bread products. The most famous are the cookies that store well. Corrosion can occur in a variety of ways, indicating visible growth on the surface, gas production leading to product destruction, or significant promotion and flavor change from fungus production. Therefore, it is unfortunate that the mushroom degradation of bread products has received more attention. The sources of fungi in the bath and the most important factors that control the decay of the cake products made have been identified. Drinking is a major obstacle to the safe life of many high and medium bath products. Alcohol damage losses have caused businesses to lose alternative currency. Therefore, how to manage sustainable growth and extend the shelf life of bread products is an important requirement for the selected industry where global demand for food is increasing. Other methods such as good sanitation in the bakery and if you need heat treatment of the boxes or better is the best option.

Further research on natural storage and maps is required for the safety of these products. The use of antifungal reagent as a natural substance, i.e., klova powder contains many chemical compounds, but the main bioactive is eugenol. Lemongrass juice also has many chemical compounds, but citral is an important

indicator of increased antifungal activity. Cardamom powder also shows significant activity against fungi. Orange extract is also high in chemicals, but limonene is an important ingredient in these extracts. Eating oil also shows antifungal activity. All these natural reagents show resistance against the growth of fungal spores. Chemicals are harmful to human health, so the use of natural ingredients helps maintain a person's health. It has no effect on the taste of food. Therefore, by using the above natural ingredients, we can manage the fungal damage of this cooking product.

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