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A 14-Day Mycological Study of the Shel Life of Bread Commonly Consumed Within the Federal Polytechnic, Mubi Community

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Abstract

This project work which is all about the fungal infestation of bakery product (Bread), explain the kind or type of fungal organisms that are responsible for the spoilage of bread. The infestation of fungal organism on bread depends on the kind and number of the agent present and the environmental conditions which they are stored in. This statement makes it clear that micro-organism infest foods having suitable environment condition for their microbial activity. This project work is centered on knowing the fungal micro-organisms that infests bread which entails the isolation and identification of the fungal organisms, the source of contamination a known standardized system called hazard analysis critical control point which ensures hygienically produced breads. The material and method include the collection of bread samples from three different bakeries in Mubi town the method used is the isolation techniques and the identification of fungi by macroscopic observation, wet mount, and light microscopy. The result obtained were gotten by counting the colonies grown on the culture medium (Potato dextrose agar) the organisms were counted and identified using a "pictorial guide for fungal identification" some fungal microorganisms viewed under the microscope are; Rhizopus spp, Mucor spp & Aspergillus spp.

Key Words: Fungi, Bread, Shelf life, Spoilage, Mycology.

Introduction

Food can be contaminated with spoilage on pathogenic micro-organism (micro-organism). Infestation of these organisms spoils the product so that it is not fit to eat it also damage the test so that it is not desirable to eat. The concept of food infestation of micro-organism is based on the consideration for micro-organism of consumption (Pitt, & Hocking, 1977).

Infestation is defined as the over presence alone has ted to its growth and microbial activities. Pathogenic organism however can cause food poisoning (Joy, 1996; Kocic–Tanackov, *et al.,* 2013). Food poisoning can arise through infestation of food by organism (micro-organism) which secretes toxins which be their consumed (Levic, *et al.,* 2009).

The cost of micro-organism infested bread resulting to spoilage is great with loss of money due to wastage of the raw materials (Brul, J.B. & Coote, P. 1999; De Boer, & Beumer 1999; Jay, *et al.,* 2005).

Also considering the usefulness of the product in the community with management of money being spent. It is being bought and consumed directly without further preparation done. Effect of contaminated bread in the society can lead to an endemic of gastro-intestinal disorder. Where by that particular environment that consume the contaminated bread become affected there by developing stomach upset (Scholte, 1996; Wogan, 2015). As a result of this a lot of consideration should be given to bread that are sent into the community to be sold and is done by the standardized system called HACCP i.e. (hazard, analysis, critical, control, point). This method is now generally considered as a choice for ensuring safety of food (jay, 1996: De Boer & Beumer, 1999) hazard analysis critical control point involves identification of places in the production process where hazard could occur i.e., the CCP (critical control point) and putting monitoring procedures in place to prevent these hazards occurring even with the system in place. Samples still need to test for the presence of micro-organisms (Samet, & Spengler, 2003; Boor, K. & Fromm, H. 2006; Nielsen, 1999; Cook, & Johnson, 2009).

Detection of most micro-organism requires growth of the organisms on selective media which can take a number of days from isolation to identification. These methods are sensitive and gives qualitative information on the number and nature of the micro-organism present in a food. However conventional methods require several days to produce result because they rely on the ability of the micro-organism to multiply to visible colonies (Brown, & Wray, 2014).

Preservatives are added to retain onto increase the shelf life by preventing the

micro-organism responsible for the contamination of that food. There alternatives to preservatives have brought also (Hammond, *et al.,*2015). The chief type of microbial spoilage of bread as a result of organism. Infestation will also be studied which is moldiness rapines is another type of bread spoilage but unfortunately it won't be studied due to the fact that it is caused by bacteria organisms and as a result is not in the scope of the study for this research work. Also, a spoilage not commonly called CHALKY BREAD caused by a yeast life fungus, will be diseased (Diaz, 2005).

Bakery products, like bread has become an important staple food in many countries. Cereals and bakery product serve as valuable source of nutrient in the diet of people. The provide most of calories. Bread provides nutrients such as carbohydrate, protein, lipids, vitamin and minerals. A variety of bread are found in the market (Flannigan, *et al.,*1991; Hamad, 2012). Bread are usually called sick man's diet or poor man's diet. But it has become essential food item for a vast majority of the whole population. Bread is made by mixing flours, salt, baking powder, and some other ingredients which follows by baking (Dilbaghi N. & Sharma S. (2007).

AIM

The research work is aimed at studying the spoilage of bread over a period of 14 days.

SPECIFIC OBJECTIVES

I. To identify the common fungi responsible for the spoilage of bread.

II. To ascertain the average shelf-life of bread produced and sold in Mubi.

LITERATURE REVIEW

Food Spoilage

Food spoilage is a metabolic process that causes foods to be undesirable or unacceptable for human consumption due to changes in sensory characteristics. Spoiled foods may be safe to eat, i.e., they may not cause illness because there are no pathogens or a toxin present, but changes in texture, smell, taste, or appearance cause them to be rejected. Some ecologists have suggested these noxious smells are produced by microbes to repulse large animals, thereby keeping the food resource for themselves (Brul, J.B. & Coote, P. 1999; De Boer, & Beumer 1999; Jay, *et al.,* 2005). Food loss, from farm to fork, causes considerable environmental and economic effects. The USDA Economic Research Service estimated that more than ninety-six billion pounds of food in the U.S. were lost by retailers, foodservice and consumers in 1995. Fresh produce and fluid milk each accounted for nearly 20% of this loss while lower

percentages were accounted for by grain products (15.2%), caloric sweeteners (12.4%), processed fruits and vegetables (8.6%), meat, poultry and fish (8.5%), and fat and oils (7.1%) (Kantor et al., 1997). Some of this food would have been considered still edible but was discarded because it was perishable, past its sell-by date, or in excess of needs. There are also environmental and resource costs associated with food spoilage and loss. If 20% of a crop is lost, then 20% of the fertilizer and irrigation water used to grow that crop was also lost. Shelf life of a food is the time during which it remains stable and retains its desired qualities (Brul, J.B. & Coote, P. 1999; De Boer, & Beumer 1999; Jay, *et al.,* 2005).

Food spoilage microorganisms

Chemical reactions that cause offensive sensory changes in foods are mediated by a variety of microbes that use food as a carbon and energy source. These organisms include prokaryotes (bacteria), single-celled organisms lacking defined nuclei and other organelles, and eukaryotes, single-celled (yeasts) and multicellular (molds) organisms with nuclei and other organelles. Some microbes are commonly found in many types of spoiled foods while others are more selective in the foods they consume; multiple species are often identified in a single spoiled food item but there may be one species (a specific spoilage organism, SSO) primarily responsible for production of the compounds causing off-odors and flavors. Within a spoiling food, there is often a succession of different populations that rise and fall as different nutrients become available or are exhausted. Some microbes, such as lactic acid bacteria and molds, secrete compounds that inhibit competitors (jay, 1996: De Boer & Beumer, 1999).

Spoilage microbes are often common inhabitants of soil, water, or the intestinal tracts of animals and may be dispersed through the air and water and by the activities of small animals, particularly insects. It should be noted that with the development of new molecular typing methods, the scientific names of some spoilage organisms, particularly the bacteria, have changed in recent years and some older names are no longer in use. Many insects and small mammals also cause deterioration of food but these will not be considered here (Flannigan, *et al.,* 1991).

Yeasts

Yeasts are a subset of a large group of organisms called fungi that also includes molds and mushrooms. They are generally single-celled organisms that are adapted for life in specialized, usually liquid, environments and, unlike some molds and mushrooms, do not produce toxic secondary metabolites (Dilbaghi N. & Sharma S. (2007). Yeasts can grow with or without oxygen (facultative) and are well known for their beneficial

fermentations that produce bread and alcoholic drinks. They often colonize foods with a high sugar or salt content and contribute to spoilage of maple syrup, pickles, and sauerkraut. Fruits and juices with a low pH are another target, and there are some yeasts that grow on the surfaces of meat and cheese (Flannigan, *et al.,*1991; Hamad, 2012).

There are four main groups of spoilage yeasts: *Zygosaccharomyces* and related genera tolerate high sugar and high salt concentrations and are the usual spoilage organisms in foods such as honey, dried fruit, jams and soy sauce. They usually grow slowly, producing off-odors and flavors and carbon dioxide that may cause food containers to swell and burst. *Debaryomyces hansenii* can grow at salt concentrations as high as 24%, accounting for its frequent isolation from salt brines used for cured meats, cheeses, and olives. This group also includes the most important spoilage organisms in salad dressings. *Saccharomyces* spp. are best known for their role in production of bread and wine but some strains also spoil wines and other alcoholic beverages by producing gassiness, turbidity and off-flavors associated with hydrogen sulfide and acetic acid. Some species grow on fruits, including yogurt containing fruit, and some are resistant to heat processing (Flannigan, *et al.,*1991; Hamad, 2012).

Candida and related genera are a heterogeneous group of yeasts, some of which also cause human infections. They are involved in spoilage of fruits, some vegetables and dairy products (jay, 1996: De Boer & Beumer, 1999).

Dekkera/Brettanomyces are principally involved in spoilage of fermented foods, including alcoholic beverages and some dairy products. They can produce volatile phenolic compounds responsible for off-flavors (Diaz, 2005).

Molds

Molds are filamentous fungi that do not produce large fruiting bodies like mushrooms. Molds are very important for recycling dead plant and animal remains in nature but also attack a wide variety of foods and other materials useful to humans. They are well adapted for growth on and through solid substrates, generally produce airborne spores, and require oxygen for their metabolic processes (Samet, & Spengler, 2003; Boor, K. & Fromm, H. 2006; Nielsen, 1999; Cook, & Johnson, 2009).

Most molds grow at a pH range of 3 to 8 and some can grow at very low water activity levels (0.7–0.8) on dried foods. Spores can tolerate harsh environmental conditions but most are sensitive to heat treatment. An exception is *Byssochlammys,* whose spores have a D value of 1–12 minutes at 90ºC (Brown, & Wray, 2014). Different mold species have different optimal growth temperatures, with some able to grow in refrigerators. They have a diverse secondary metabolism producing a number of toxic

and carcinogenic mycotoxins. Some spoilage molds are toxigenic while others are not (Boor, K. & Fromm, H. 2006; Nielsen, 1999).

Spoilage molds can be categorized into four main groups: *Zygomycetes* are considered relatively primitive fungi but are widespread in nature, growing rapidly on simple carbon sources in soil and plant debris, and their spores are commonly present in indoor air. Generally, they require high water activities for growth and are notorious for causing rots in a variety of stored fruits and vegetables, including strawberries and sweet potatoes. Some common bread molds also are *zygomycetes*. Some *zygomycetes* are also utilized for production of fermented soy products, enzymes, and organic chemicals. The most common spoilage species are *Mucor* and *Rhizopus*. *Zygomycetes* are not known for producing mycotoxins but there are some reports of toxic compounds produced by a few species (Nielsen, 1999; Cook, & Johnson, 2009).

Penicillium and related genera are present in soils and plant debris from both tropical and Antarctic conditions but tend to dominate spoilage in temperate regions. They are distinguished by their reproductive structures that produce chains of conidia. Although they can be useful to humans in producing antibiotics and blue cheese, many species are important spoilage organisms, and some produce potent mycotoxins (patulin, ochratoxin, citreoviridin, penitrem) (Brown, & Wray, 2014). *Penicillium* spp. cause visible rots on citrus, pear, and apple fruits and cause enormous losses in these crops. They also spoil other fruits and vegetables, including cereals. Some species can attack refrigerated and processed foods such as jams and margarine. A related genus, *Byssochlamys*, is the most important organism causing spoilage of pasteurized juices because of the high heat resistance of its spores (Samet, & Spengler, 2003; Boor, K. & Fromm, H. 2006).

Aspergillus and related molds generally grow faster and are more resistant to high temperatures and low water activity than *Penicillium* spp. and tend to dominate spoilage in warmer climates. Many aspergilla produce mycotoxins: aflatoxins, ochratoxin, cyclopiazonic acid. Aspergilli spoil a wide variety of food and nonfood items (paper, leather, etc.) but are probably best known for spoilage of grains, dried beans, peanuts, tree nuts, and some spices (Pitt, & Hocking, 1977).

Other molds, belonging to several genera, have been isolated from spoiled food. These generally are not major causes of spoilage but can be a problem for some foods. *Fusarium* spp. cause plant diseases and produce several important mycotoxins but are not important spoilage organisms. However, their mycotoxins may be present in harvested grains and pose a health risk (Cook, & Johnson, 2009).

Method

Sampling Technique

Loafs of bread for the research were purchased directly from the bakeries (to ensure they were all baked the same day and to reduce contamination due to market handling).

The stratified sampling method was used to select bakeries that were used for the research.

Sample Size

A total of ten (5) loaves of bread were purchased from five (3) different bakeries, making it a total of 15 loaves of bread, 3 loaves (From each bakery) were purchased on day one of the experiment. After four days, another three loafs were purchased, after seven days, another three were purchased, after 10 days, another three were purchased and finally, after fourteen days another three loafs were purchased.

Experimental Procedure

The loaves were kept at normal room temperature for the period of days under investigation and one loaf each was used for the experiment.

Sample preparation

One thousand mills of distil water (1 litter) was added to a sterile blender and a loaf of bread cut into pieces added into the blender and blended to have a homogenous mixture. One gram of the blended bread was then transferred into sterile test-tubes for serial dilution.

Serial Dilution

1g from of each sample was taken and used for serial dilution to reduce microbial load. Dilution 3 and 7 were used for the culture.

Media Preparation

For the culturing of the fungi, Potato Dextrose Agar was used and the preparation was done according to the manufacturer's procedures.

Culturing

Potato Dextrose Agar (PDA) was prepared and used for the culturing of the fungi. The direct pour plate method was used for the inoculation into the prepared agar and was grown under room temperature (and observed) for a period of 48 hours to 7 days.

Macroscopic Observation of the Fungi

Observations of fungal colonies were done from 24 hours to 7 days period and all the observations made were recorded as shown below:

Table **Error! No text of specified style in document.**.1: Macroscopic Observation of Cultures

Sample	Shape of Colony	Color of Colony	Size of Colony	Number of Colonies	Nature of Colonies
A					
B					

Microscopic Observation

The direct wed mount was used for the identification of the fungi using the binocular light microscope at X 10 and X 40 magnification and the following features observed.

Table **Error! No text of specified style in document.**.2: Microscopic Observation of Fungi

Sample	Fruiting Body	Branching	Rhizoids	Color	Nature of Spores
A					
B					

RESULT

Table 3: Fungal Macroscopic and Microscopic Observations

Plate	Plate Colony	Hypha	Septa	Rhizoids	Fruiting bodies	Spores
A	Brown, Powdery colonies	Showed branching	Unsepted mycelia	Present at nodes	Sporangia	Numerous, brownish
B	Yellowish Green Powdery growth	No branched	the hypha is septet	No rhizoids	Conidiophore Yellowish brown	Conidia are compact, brownish black.
C	Cottony whitish	Highly branched	Unsepted mycelia	No rhizoids	Sporangiophores	Numerous sporangiospores
D	Powdery blackish	Slight branching	Unsepted mycelia	No rhizoids	Conidiophore blackish	Conidia are numerous and black in color

The above shows the physical observations made of the colony appearance on the culture plates (petri dishes) and the microscopic observations made under the microscope.

The observations made were compared with standard fungal charts for identification of the fungi.

Table 4: Colony counts for sample A for 14 days

S/N	Days	Colony counts
A	Day 1	Too numerous
B	Day 4	Too Numerous
C	Day 7	12 X 4 = 48
D	Day 10	8 X 4 = 32
E	Day 13	No Growth

Table 5: Colony counts for Sample B for 14 days

S/N	Days	Colony counts
F	Day 1	Too numerous
G	Day 4	18 X 4 = 72
H	Day 7	15 X 4 = 60
I	Day 10	10 X 4 = 40
J	Day 13	No Growth

Table 6: Colony counts for Sample C for 14 days

S/N	Days	Colony counts
K	Day 1	Too numerous
L	Day 4	Numerous
M	Day 7	12 X 4 = 48
N	Day 10	10 X 4 = 40
O	Day 13	No Growth

Table 7: Physical observation of bread over a period of 14 days

Days	Bread	Color	Odor	Fungal Colonies
Day 1	Sample A	Normal	Normal	None
	Sample B	Normal	Normal	None
	Sample C	Normal	Normal	None
Day 4	Sample A	Normal	Normal	None
	Sample B	Normal	Slight change in odor	None
	Sample C	Normal	Normal	None
Day 7	Sample A	There's Color change	Smell of spoilt bread	Slight growth of Fungi
	Sample B	There's Color change	Smell of spoilt bread	Slight growth of Fungi
	Sample C	There's Color change	Smell of spoilt bread	Slight growth of Fungi
Day 10	Sample A	Heavy color change	Smell of spoilt bread	Heavy growth of Fungi

	Sample B	Heavy color change	Smell of spoilt bread	Heavy growth of Fungi
	Sample C	Heavy color change	Smell of spoilt bread	Heavy growth of Fungi

DISCUSSION

This research work was carried out using freshly purchased bread different brands left after several days of production (14 days). Annur special bread, Bakin ka ya gaya maka & Hyalanda special bread were used during his study. The bread was left in its wrapper to undergo spoilage at room temperature. This was done to determine if contamination occurred at site of production or during post production operations. Contamination of the products mostly occur after baking and the wrapping bags also constitute a source of contamination. This is because the high temperature attained during baking procedure is sufficient to kill all fungal spores, (Scholte, 1996; Wogan, 2015). The color change of the bread was seen viewed by the use of the naked eye, the odor passive by the nose the Appearance of the fungal growth was viewed with the naked eye. The samples were cultured on PDA incorporated antibiotic (to inhibit the growth of bacteria) after 14 days of purchase. After culturing, some relative level of contamination was observed. The book "a pictorial guide to fungi identification" was used for the identification of the fungi. All three breads showed very similar patterns in spoilage with same organisms. *Mucor* growth was the highest, spreading all over the three sample breads used. Other species of fungi are *Rhizopus niger* and *R. flavus*. The samples were examined on daily basis as shown in Table 5. It was noted that no physical sign of spoilage and fungal colonies were observed until after the fifth day of storage (Table 5). After the fifth day White, Yellow, Green, Black color changes (showing fungal colonies) were observed on the bread from the 5^{th} day of storage to the 14^{th} day, a slightly state odor was observed from the 4^{th} day to the 14^{th} day of storage and a high Number of fungal colony were observed from the 6^{th} day of storage to the 14^{th} day of storage.

CONCLUSION

Conclusively the overall degree of contamination determines the quality of the sample and form all indications. Bread spoilage be attributed to poor handling and storage condition since the temperature of 230^{o}C attained during the baking procedure would have killed all mold spores, (Scholte, 1996; Wogan, 2015). There should be prompt and adequate cooling of the bread loaves before wrapping so as to reduce condensation of moisture beneath the wrapper since heavy contamination after baking is prone to heavily laden with mold spore, a long cooling time and considerable air circulation all favor mold growth, incorporation into bread dough of some mycostatic chemicals could also control bread spoilage.

RECOMMENDATIONS

Regulatory bodies should be put in place to ensure the regulation of bread sold after overstaying the period of 3 days.

In order to reduce on probably eliminate this incidence of contamination and spoilage of bread, bakeries and other food industries should maintain adequate cleaning and sanitation of equipment

This is essential for reasons of producing an uncontaminated acceptable produce. Since bread has become an essential part of the Nigerian diet which is also relatively cheap compared to other bakery product. The world health organization (WHO) or the government could assist in ensuring the production of uncontaminated bread. This could be done by setting up microbiological standard and dispiriting sanitary inspectors to different bakeries and other food industries without notifying them. They will then examine the personal hygiene and working conditions of the workers. This will go a long way to ensure that their finished product will not be hazardous to human health and the population at large.

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