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Mycoflora of Slice breads causing their spoilage

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Abstract: *Breads are an important part of balanced diet. It is a good source of nutrients, such as macronutrients and micronutrients that are essential for human health. Breads are subjected to fungal attack causing their spoilage. In the present investigation ten filamentous fungi viz. Penicillium chrysogenum, Mucor sp. Rhizopus sp., Aspergillus sp. Fusarium sp., Alternaria sp., Phoma sp., Curvularia sp., Cladosporium sp., Eurotium sp., and one yeast Pitchia burtonii were isolated. These are involved in spoilage of bread due to improper handling and improper sanitation. Therefore its importance and commercial value is lost due to improper handling. Spoilage of these bakery products may constitute a health risk in food and may cause a mild to severe food illness. The percentage incidence of Mucor sp., Rhizopus sp., Curvularia sp., Aspergillus sp. Fusarium sp. and Penicillium chrysogenum was highest.*

Key Words: *Mycoflora, Slice bread, Spoilage, Filamentous fungi, Yeast*

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I. Introduction

Bread is a stable food prepared by cooking a dough of flour and water and some additional ingredients. Salt, fat and leave ling agents such as Yeast *(Saccharomyces cerevisiae*) and baking soda are common ingredients. Bread may contain milk, egg, sugar, spice, fruit, vegetables, nuts or seeds.

Fresh bread is prized for its tastes, aroma, quality, appearance and texture. There are several different types of bread prepared around the world, viz., white bread, brown bread, whole meal bread, wheat germ bread, whole grain bread, Roti or Chapatti, Granary bread, Rye bread, unleavened bread or matzo, sourdough bread, flat bread, hemp bread, crisp bread etc.

In breads the amount of flour is always stated as 100% and the amounts of the rest of the ingredients are expressed as a percent of that amount by weight. The grains used in flour making provides starch and proteins needed to form bread. The protein content of the flour is the best indicator of the quality of bread. In addition to starch, the wheat flour contains three water soluble proteins viz., albumin, globulin and proteases, and two water insoluble protein, glutenin and gliadin. When flour is mixed with water, the water soluble proteins dissolve, leaving the glutenin and gliadin to form the structure of the resulting bread. Ascorbic acid, hydrochloride, sodium metabisulphate, ammonium chloride, various phosphates, amylase and protease are commonly used as ingredients to improve the quality of bread. In addition to these, three natural phenolic glucosides viz., secoisolariciresinol, p-coumaric acid glucoside and Ferulic acid glucoside are also found in commercial breads.

The composition of a typical white and wheat slice bread can be summarized as follows:

Nutritional value per 100gm(3.5oz)	White bread (typical bread)	Brown bread (Whole wheat bread)
Energy	1,113 KJ(266k.cal)	1,034KJ(247kcal)
Carbohydrates	51g	41g
Dietary fiber	2.4g	7g
Fat	3g	3g
Protein	8g	13g
Thiamine(Vit.B1)	0.5mg(43%)	0.4mg(35%)
Riboflavin(Vit.B2)	0.3mg(25%)	0.2mg(17%)
Niacin(Vit.B3)	4mg(27%)	4.7mg(31%)
Folate(Vit.B9)	111µg(28%)	50µg(13%)
Choline	14.6mg(3%)	26.5(5%)
Vitamin K	3.1µg(3%)	7.8µg(7%)
Calcium	151mg(15%)	107mg(11%)
Iron	3.74mg(29%)	2.43mg(19%)
Magnesium	23mg(6%)	82mg(23%)

Potassium	100mg(2%)	248mg(5%)
Sodium	681mg(45%)	472mg(31%)

Percentages are relative to US recommendation for adult

Slice bread is subjected to microbial attack that cause spoilage of nutrients by way of decomposition. Carbohydrates (starch), fats and proteins are the basic constituents of slice bread. Hence slice bread is susceptible to degradation by a great many species of fungi, yeasts and bacteria. The microorganisms causing breakdown of carbohydrates, fats and proteins vary with the environment. Under aerobic conditions a wide range of saprophytic fungi such as *Mucor, Rhizopus, Eurotium, penicillium, Aspergillus, Cladosporium, Auriobasidium, Thermoascus, Monila*, etc. colonize the bread. Growth of amylolytic, lipolytic and proteolytic fungi helps in the decomposition and spoilage of bread. Among different groups of microbes, fungi have an edge over others in initiating the breakdown of solid substrata, partly because of their superior enzymatic equipment and partly because of their filamentous growth.

Spoilage of Food commodities: *Food spoilage can be defined as "any sensory change (tactile, visual, olfactory or flavour)" which the consumerconsiders to be unacceptable.* Spoilage of food commodities 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 (Burkepile *et al*., 2006) [1].

Microorganisms involved in food spoilage: Soil is an obvious source of contamination, as a diverse community of microorganisms viz. bacteria, yeasts, molds, actinomycetes, etc. thrive in most soils and can grow to very large numbers. Direct contamination with soil microorganisms occurs during production and harvesting. Indirect contamination with soil occurs through the deposition of wind-borne dust particles. Wind-borne mold spores are a very common cause of mold spoilage of foods, as well as human allergies. Water can serve as a source and a vector of contamination. The nearly countless microbial genera are involved in food spoilage. These include bacteria, actinomycetes, yeasts and moulds (filamentous fungi).

Molds. *Moulds are* capable of growth across a broad range of temperatures. They are obligate aerobes with oxidative metabolism and can grow across the range of water activity from 0.62 to nearly 1.0. Molds are the most common food spoilage microorganisms at every step of the food chain from field crops to consumer food products. They are also capable of spoiling bottled mineral water (Criado, Pinto, Badessari and Cabral, 2005) [2]. Representative genera of food spoilage molds are *Penicillium, Aspergillus, Rhizopus, Mucor, Geotrichum, Fusarium, Alternaria, Cladosporium, Eurotium,* and *Byssochlamys.*

Yeasts: Yeasts can be described in two broad categories: fermentative and oxidative. Yeasts are generally mesophilic and grow best above water activity values of 0.9. Both molds and yeasts grow at slower rates than bacteria. Spoilage of perishable foods by these microorganisms often indicates that the food has simply been "stored
too long." *Fermentative yeasts,* commonly known spoilage yeasts are facultatively anaerobic fermentative organisms, producing ethanol and carbon dioxide from simple sugars. Some fermentative yeasts are the most osmophilic organisms known, capable of slow growth at water activity 0.60 (Martorell, Fernández-Espinar and Quereol, 2005) [3]. Representative genera include *Saccharomyces* and *Zygosaccharomyces. Oxidative yeasts.* Less are the aerobic "film yeasts" which can grow on fermented foods and metabolize organic acids and alcohols. These yeasts seem to occupy an evolutionary middle ground between fermentative yeasts and molds, possessing the morphological characteristics of yeasts and the metabolic characteristics of molds. Representative genera include *Mycoderma, Candida, Pichia,* and *Debaryomyces.*

Bacteria involved in food spoilage are grouped into following families viz.

Pseudomonadaceae. The principal genera in this family of bacteria, *Pseudomonas* and *Xanthomonas,* are Gram-negative rods, nonspore forming, psychrophilic, aerobic, and oxidase positive. They are also completely intolerant of
reduced water activity, growing in foods mostly above water activity 0.98. The addition of small amounts of solutes, such as 2% sodium chloride, will substantially restrict their growth. Pseudomonads are primary spoilage microorganisms in fresh meat, poultry, seafood, and eggs.

Neisseriaceae. This includes are Gram negative rods, nonspore forming, aerobic, and catalase positive. The spoilage genera are *Acinetobacter* (oxidase negative) and *Moraxella*(oxidase positive). Some strains of *Acinetobacter* are psychrophilic.

Enterobacteriaceae. This family of Gram-negative rods is facultatively anaerobic, fermentative, mesophilic, nonspore forming, oxidase negative, and catalase positive and is generally incapable of growth below water activity 0.95. All of the 28 genera in this family are commonly called "enteric" bacteria and ferment glucose with the production of acid and gas. A subset of this family, containing about half of the genera, is commonly called "coliform" bacteria, as established by their ability to ferment lactose with the production of acid and gas. Representative spoilage genera include *Escherichia, Erwinia, Enterobacter, Citrobacter, Serratia*, and *Proteus.* Enteric bacteria are often involved in the spoilage of fresh vegetables, meat, poultry, fish, and eggs.

Micrococcaceae. The two principal genera of bacteria in this family are *Micrococcus* and *Staphylococcus*. They are Gram positive, spherical, catalase positive, and mesophilic. *Micrococcus* is oxidative, growing on glucose without the production of acid or gas, while *Staphylococcus* is fermentative, producing both acid and gas from glucose. *Staphylococcus* is osmotolerant. Both the genera are commonly involved in the spoilage of fresh produce and processed meat, poultry, and seafood.

Lactic Acid Bacteria. All members of this group are Gram positive, catalase negative, microaerophilic or facultatively anaerobic, and fermentative. Homofermentative lactics ferment glucose with the production of lactic acid only. Heterofermentative lactics ferment glucose with the production of lactic acid, carbon dioxide, and ethanol or acetic acid. *Lactobacillus* is rod shaped, while *Streptococcus, Lactococcus, Leuconostoc, Enterococcus*, and *Pediococcus* are spherical. The "lactics" are generally mesophilic and grow at water activity values above 0.9. The growth of lactics in meat, vegetable, and dairy products is used to advantage to produce fermented foods such as salami, sauerkraut, and cheese. However, the growth of these bacteria in the same fresh foods, such as luncheon meats, vegetable salads, and fluid milk, constitutes spoilage.

Coryneforms. These microorganisms, of relatively minor importance in food spoilage, are sometimes involved in cheese spoilage. Representative genera are *Corynebacterium* (facultatively anaerobic) and *Brevibacterium* (aerobic). Both are Gram positive and catalase positive. Their sources of contamination are usually soil, animals, or humans.

Spore-forming Bacilli. There are three major genera of bacterial sporeformers important in food spoilage – *Bacillus, Clostridium*, and *Alicyclobacillus*. All are Gram-positive rods and are generally mesophilic or thermophilic. Because these genera produce heat-resistant endospores, they are the predominant spoilage microorganisms in pasteurized foods in which all vegetative cells have been killed and in improperly sterilized foods.

Bacillus species are aerobic or facultatively anaerobic, catalase positive, and generally not osmotolerant.While most species are mesophilic, individual species cover the entire temperature spectrum for food spoilage. *Bacillus cereus* can spoil pasteurized milk (psychrotrophic), *B. subtilis* can spoil bakery products (mesophilic), and *B. stearothermophilus* can spoil canned foods (thermophilic).

Clostridium species are obligate anaerobes, catalase negative, and not osmotolerant. They are typically involved in the spoilage of foods that have a highly negative O/R potential, such as canned or vacuum-packaged foods. The principal spoilage species are *C. sporogenes* and *C. butyricum* (mesophilic) and *C. thermosaccharolyticum* (thermophilic).

Alicyclobacillus species were discovered in the 1960s and originally classified as *Bacillus* spp. First isolated from acid hot springs in Yellowstone Park, these bacteria typify a significant new ecological grouping of microorganisms called "extremophiles."

Quite unlike all other food borne bacteria, alicyclobacilli are extreme acidophiles, growing within a pH range of about 2.0–6.0. They are moderate-to-obligate thermophiles, catalase positive, and microaerophilic. Like pseudomonads, the alicyclobacilli cannot tolerate osmotically increased environments, that is, below water activity of 0.98. They have evolved to grow in acid and hot water, and it is these types of foods that they can spoil. The principal spoilage species *A. acidoterrestris* is sometimes involved in the spoilage of pasteurized fruit or vegetable juices that have been improperly cooled or stored at relatively high temperatures, above 30◦C.

Mechanism of Microbial food spoilage: The microbiological spoilage of foods occurs because of the biochemical activity of microorganisms as they grow in the food. Food spoilage is usually an indicator that a food has been improperly handled or stored too long. Such mishandling could permit the growth of food borne pathogens that could cause illness or death if the food were to be consumed. Since food borne pathogens do not typically give an organoleptic indication of their presence, the organoleptic changes caused by spoilage microorganism serve as a warning to the consumer that the food could be unsafe for consumption. It can be argued that spoilage microorganisms routinely protect millions of people from food borne illness.

The protective feature of food spoilage does not always protect the consumer from the threat of foodborne illness. The main reason for this fact is that microbiological spoilage of foods is not organoleptically detectable until a substantial growth of the spoilage organism has occurred. Typically, the threshold level for observation of food spoilage by odor, taste, or sight is not reached until the spoilage microflora exceeds about 107 organisms/g of food. Spoilage characteristics develop in food as microorganisms digest the food to support their growth. The digestion of sugars, complex carbohydrates, proteins, and fats can all produce undesirable effects in the food if the spoilage microorganisms grow to significant levels. Spoilage of food occurs due to certain metabolic activities of microbes viz. *Sugar fermentation with acid production, Sugar fermentation with gas production, Protein hydrolysis, Digestion of complex carbohydrates, Lipolysis, Oxidation of organic acids and alcohols, Guaiacol production and Surface growth.*

Quorum Sensing: Some of the spoilage mechanisms do not involve the steady production and secretion of enzymes as the microbial population increases. The phenomenon called quorum sensing has been discovered to be responsible for many of the effects of large microbial populations (Smith, Fratamico and Novak, 2004) [4]. Quorum sensing has been shown to be active in the production of toxins, invasive factors, dental plaque, biofilms, bioluminescence, bacteriocins, and even food spoilage (Cotter, Hill, & Ross, 2005; Rasch *et al*., 2005; Gram *et al*., 2002) [5, 6, 7].

Quorum sensing depends upon the synthesis of a biochemical signal molecule, followed by its accumulation in the growth environment and recognition by other cells of the same microbial species. *N*-Acylhomoserine lactones (AHLs) produced by Gram-negative bacteria are the most common quorum-sensing signal.

Intrinsic Factors to Control Microbiological Spoilage: There are a number of inherent, or intrinsic, food properties, such as water activity, pH, preservative compounds, and O/R potential, that affect the type and rate of microbial spoilage.

***Water Activit:* (*a*w):** The *a*w can be determined manometrically by dividing the vapor pressure of the food by the vapor pressure of water. It can be estimated mathematically for individual solutes by dividing the moles of water by the moles of water plus the moles of solute. *a*w values will range from 0 to 1.0.

The influence of a solute on water activity varies inversely with its molecular or ionic size. Therefore, smaller molecules or ions will be more effective than larger molecules in reducing water activity in food formulations. Sodium chloride (ionic weight = 29.25) is theoretically 11.7 times more effective than sucrose (molecular weight = 342) on an equal weight basis in reducing water activity.

Microorganisms vary greatly in their ability to grow in foods with increased osmotic pressure or reduced water activity values.
The type of spoilage organism likely to spoil a particular food can be estimated by the determination of the food's water activity. The water activity value of bread is 0.94 and of wheat flour is 0.60.

pH: The pH value of foods is another important intrinsic value that determines what types of microorganisms can spoil a food. In general, many kinds of food borne spoilage microorganisms can grow collectively over most of the pH range, from 0.5 to 11.0. Most food borne bacteria can grow in the pH range of 4.5–9.0. Most foods range in pH from slightly acidic to strongly acidic.

Chemical Preservatives: In addition to the microbiostatic action of their pH effect, organic acids exert various internal metabolic effects. Only undissociated acids, however, can enter the microbial cell by migrating through the cytoplasmic membrane. Therefore, the preservative activity of organic acids is dramatically affected by the pH of the food. Some of the commonly used preservatives occur naturally, especially in acidic foods. Therefore, it is possible that they have been used as food preservatives since antiquity. *Sorbic acid, Propionic acid, Benzoic acid, Methyl and propyl parabens etc.* are important chemical preservatives.

Oxidation–Reduction Potential: The oxidation–reduction potential of a food is often referred to as the O/R potential or the Eh of the food. It is expressed in a range with a maximum Eh value of +816 mV (highly oxidized) and a minimum Eh value of −421 mV (highly reduced). The positive Eh values favor the growth of aerobic microorganisms because these can grow in the presence of oxygen and often use oxidative metabolic processes (which would tend to further increase the food Eh). Negative Eh values favor the growth of anaerobic icroorganisms, whose growth and use of fermentative metabolic pathways will further reduce the food's Eh value.

Fungal association with bakery products, the slice bread and its control by preservatives have been reviewed by several authors viz. Saranraj and Geetha (2012), Francesca Vaberio *et al*., (2009), Hunt and Robbins (2009), Anon (2000), Baur (2001) [8-12].

Microbial spoilage of bakery products has been studied by Rachel Needham (2004), Bailey and Holy (1993), Seiler (2000), Dickson (2001) [13-16].

Spoilage of bakery products by Yeast(*Trichosporon variable*, *Saccharomyces*, *Pichia*, *Zygosaccharomyces*) has been reviewed by Legan and Voysey (1981) [17]. Spoilage of bakeries by molds has also been reviewed by Membre *et al*., (2001), Malkki and Rauha (2000), Hickey (1998), Knight and Menlove (2006) and Jarvis (2001) [18-22].

Physical factors influencing the growth of microbes on bakery products have been studied by Ponte *et al*., (1993) [23], Abellana *et al*., (1999) [24], Frazier and Westhoff (1978) [25], Chamberlain (1993) [26], Abellana *et al*., (2001) [27], Mariona Arroyo *et al*., (2008) [28], Vytrasova *et al*., (2002) [29], and Guynot *et al.*, (2003) [30]. Samapundo *et al*., (2010) [31] evaluated the effect of NaCl on the growth of *Penicillium roqueforti* and *Aspergillus niger* at 22^0C colonizing white bread.

Slice bread provides a suitable substratum for fungi. The fungi cause complete spoilage and destruction of bread by their amylolytic, lipolytic and proteolytic activity and so it has been decided to study the role played by some dominant fungal flora in the spoilage of slice bread. The quality of bread, the physical factors of the environment helping spoilage of bread and the chemical factors greatly affect the growth and sporulation of fungi colonizing the bread. The present investigation is aimed to isolate and characterize mycoflora causing spoilage of slice bread.

II. Materials and Methods

Collection of sample

Brown and white slice breads were collected from local shops of Patna. The collected bread samples were brought in sterile polythene bags to the laboratory for analysis. The open contaminated samples were brought to the laboratory environment and maintained at room temperature 25-27^0C for the fungal growth. The growth of fungi was observed in 7 days. Slides were prepared by scrapping the products for identification of fungi.

Preparation of media

A small fragments of these fungi were transformed aseptically to Asthana and Hawker's medium 'A' consisted of 5 g glucose, 3.5g KNO3, 1.75g KH2 PO4, 0.75g MgSo4.7H2O and 15g agar agar. The media was sterilized in autoclave at 121^0C for 15 minutes. After sterilization it was allowed to cool down at about 40^0C. All the glass wares used in this study were sterilized in a hot air oven at 160^0C for 2 hours. The other materials were sterilized by autoclaving at 121^0C for 15 minutes.

About 20 ml of the media was poured into each sterilized petri dish. The media in petri dish was allowed to solidify. 1g of bread sample was mixed with distilled water and a homogenate was prepared and then added on the surface of the media and spread evenly over the surface using a sterile spreader (bend glass rod). The plates were incubated in an upright position at 30^0C for five days. The same procedure was carried out for all the samples.

The fungal count was recorded in terms of Colony Forming Units (CFU). The different types of colonies were used as inoculum to obtain pure cultures by sub culturing in Asthana and Hawker's media.

The slides were made and morphology i.e. shapes, size and structure of conidia, conidiophores, pigmentation, shape of sporangia, sporangiophores were recorded. The fungal isolates were identified and characterized following standard methods of Alexander (1999) [32], Harrigan (1988) [33], Dubey and Maheshawi (2004) [34].

The frequency of each fungal isolates was determined following formula:

$$\text{Percentage frequency} = \frac{\text{Number of observations in which a species appeared}}{\text{Total number of observations}} \times 100$$

The spoilage of bread was observed by the appearance of mould growth. Uncontaminated bread was represented by sign "A" and contaminated bread by sign "B".

III. Results

In the present investigation ten filamentous fungi viz. *Penicillium chrysogenum*, *Mucor* sp. *Rhizopus* sp., *Aspergillus* sp. *Fusarium* sp., *Alternaria* sp., *Phoma* sp., *Curvularia* sp., *Cladosporium* sp., *Eurotium* sp.

and one Yeast *Pithicia butonii* were isolated from white and brown slice breads (Table-1; Figure-1; Photograph-1 and 2; Microphotographs).

Table-1: Mycoflora of breads and their percentage frequency

Fungal flora of Slice bread	Brown bread	White bread	Percentage frequency	Number of colonies appeared	Total Number of colonies
Penicillium chrysogenum	+	+	**9.0**	**5**	**55**
***Mucor* sp.**	+	+	**12.0**	**7**	
***Rhizopus* sp.**	+	+	**12.0**	**7**	
***Aspergillus* sp.**	+	+	**10.0**	**6**	
***Fusarium* sp.**	+	+	**10.0**	**6**	
***Alternaria* sp.**	+	+	**7.0**	**4**	
***Phoma* sp.**	-	+	**5.4**	**3**	
***Curvularia* sp.**	+	+	**12.0**	**7**	
***Cladosporium* sp.**	+	+	**7.0**	**4**	
***Eurotium* sp.**	-	+	**3.6**	**2**	
Pithia butonii	-	+	**7.0**	**4**	

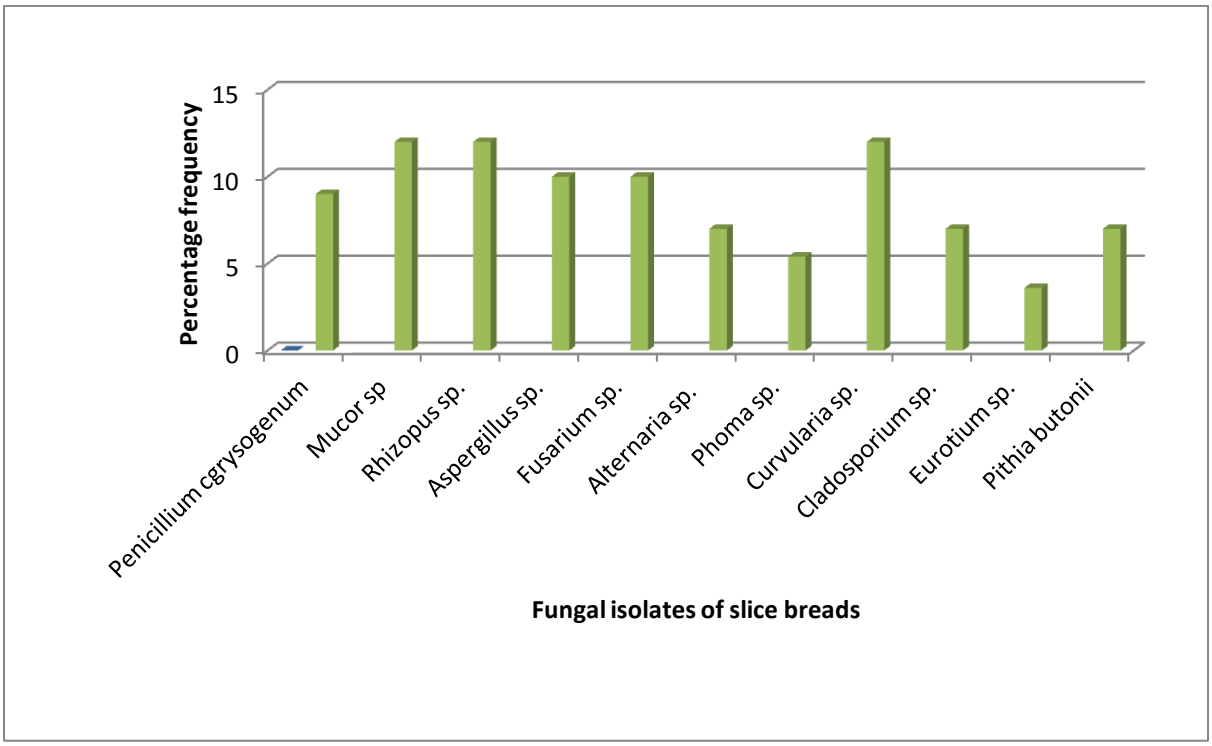
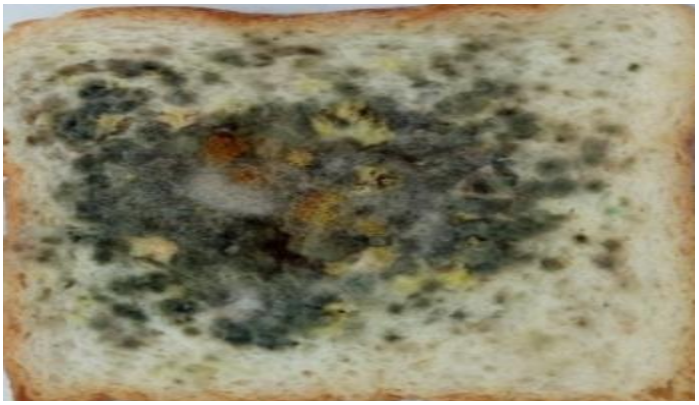
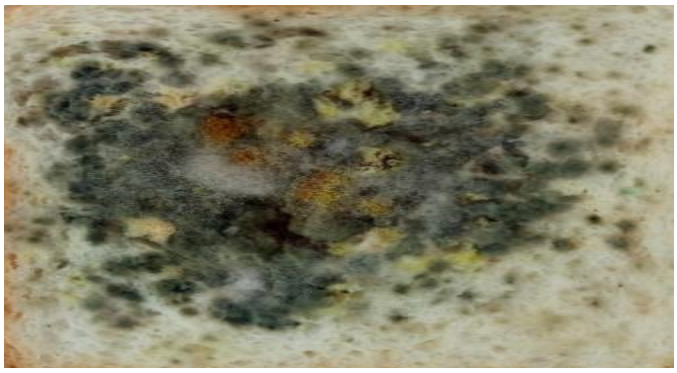


Figure-1: Percentage frequency of bread mycoflora

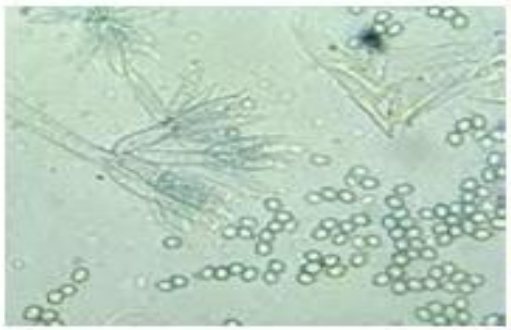


Photograph-1: Brown bread infested with spoilage fungi

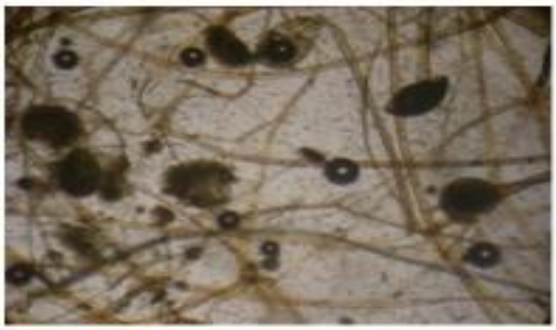


Photograph-2: White bread infested with spoilage fungi

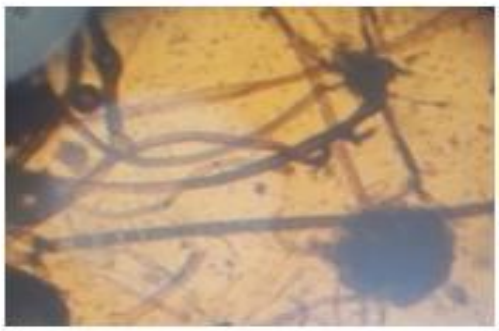
Microphotographs



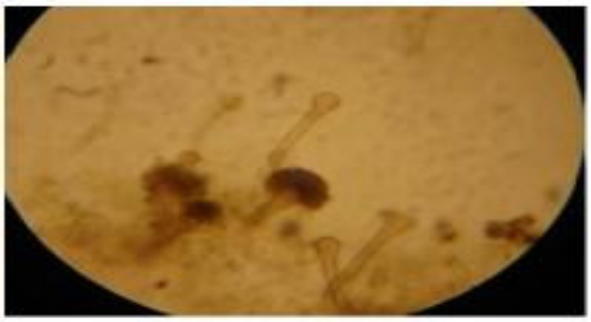
Penicillium chrysogenum



Mucor sp.



Rhizopus sp.

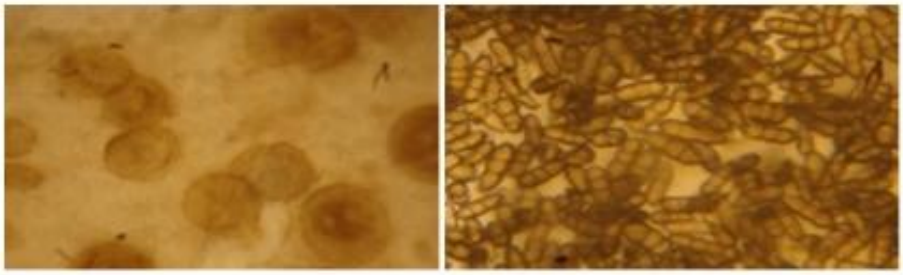


Aspergillus sp.

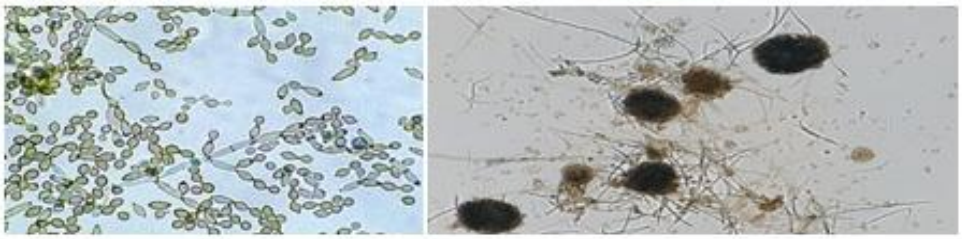
Fusarium sp.

Alternaria sp.

Microphotographs



Phoma sp. *Curvularia sp.*



Cladosporium sp. *Eurotium sp.*

Pitchia burtonii

Table-2: Bread spoilage by fungi and yeast in seven days

Bread types	Days of infestation						
	1	2	3	4	5	6	7
Brown bread	A	B	B	B	B	B	B
White bread	B	B	B	B	B	B	B

Table-3: Cultural and morphological characteristics of bread spoilage fungi

Fungal isolates	Cultural characteristics	Morphological characteristics
Penicillium chrysogenum	Large fluffy white colonies almost covering the whole surface on agar plate	Aseptate branched hyphae, enlarged at the apex to form conidophores; produced brownish black conidia in chains
Mucor sp.	Cream white/large fluffy white colonies almost covering the whole surface on agar plate	Aseptate branched hyphae; sporangium comes out directly from the hyphae without stolon or rhizoids, columella spherical.
Rhizopus sp.	Large fluffy white milky colonies which later turns black as culture ages	Aseptate hyphae with uptight sporangiophore connected by stolon and Rhizoids, dark pear shaped sporangium on hemispherical columella
Aspergillus sp.	Very common colors of colony (black and white)	Conidia borne in 360 arrangements covering the upper 2/3 of the conidiophores
Fusarium sp.	Colonies rapidly growing, wooly to colt only lemon and yellow	Hyphae septate with multicellular distinctive sickle shaped macro conidia.
Alternaria sp.	Colonies usually black on agar	Hype dark brown, branched, wide, conidiophores arise singly or

	plate	in small groups, branched flexuous, geneculated, pale to olivaceous or golden brown, smooth, thick with one or several conidial scars, conidia form in branched chain, of obcalcuate obpyriform, ovoid or ellipsoidal of often with short conical or cylindrical beak, pale to mid- gloden brown, smooth or verruculos, with upto 8 transverses and usually several longitudinal or oblique septa, black pale thick
Phoma sp.	Colonies black with spreading hyphae	Hyphae hyline, branched, septated, pycnidium globous to subglobulous, glabrous, conidia one celled, hyline.
Curvularia sp.	dark black colonies in culture	mycelium branched, septated, subhyline, dark to light brown, conidiophores erected, slightly bent, dark brown, unbranched, septated, geniculate towards apex. Conidia produce acrogenously at the tip of conidiophores and on its successive growing point. Conidia boat shape, brown, three, septed, the tired cell from the base conspicuously large, broader and darkar than the other cells, other cells lightly curve, sub-hyline with rounded apical cell and sub-hyline somewhat of conical basal cell which bears a scar indicating point of attachment to the conidiophores.
Cladosporium sp.	Olive-green to brown or black colonies on agar plate	dark-pigmented conidia, formed in simple or branching chains
Eurotium sp.	Colony characters more or less similar to Aspergillus	Sexual stage of Aspergillus
Pitchia burtonii	Colonies black, spreading	hyphae hyline, branched, septated, pycnidium globous to subglobulous, glabrous, conidia one celled, hyline.

The percentage frequency of *Mucor* sp., *Rhizopus* sp., and *Curvularia* sp. was highest (12.0% each) among the mycoflora of bread, followed by *Aspergillus* sp. and *Fusarium* sp. (10.0% each), *Penicillium chrysogenum* (9.0%), *Alternaria* sp., *Curvularia* and *Pitchia burtonii* (7.0% each) and *Phoma* sp. (5.4%). *Eurotium* sp. had lowest percentage frequency (3.6%) (Table-1; Figure-1).

In the first day of incubation brown bread did not show any sign of fungal infestation but white bread showed sign of infestation. The intensity of infestation increased in increasing incubation period. After seven days of incubation both brown and white bread showed heavy infestation of spoilage fungi (Table-2; Photograph-1 and 2).

The cultural and morphological features of mycoflora causing spoilage of slice bread have been summarized in Table-3 and Microphotographs of eleven mycoflora.

IV. Discussion

Breads are bakery products easily infected or spoiled due to the infection of fungi. They affect the quality of the product. The product shows discoloration, decrease the stored or packaging capacity of the product and some chemical and physical changes occurs in it. In the present investigation infected brown and white breads were collected, and on these bakery products eleven fungi (ten filamentous and one yeast) are commonly found. These fungi werePenicillium chrysogenum, Mucor sp. rhizopus sp., *Aspergillus, Alterneria, Fusarium, Phoma, Curvularia, Cladosporium, Eurotium* and *Pitchia burtonii*. On both the breads, molds like *Mucor* and *Rhizopus* were found to grow first which causes bread spoilage. This was followed by other fungi such as *Aspergillus, Fusarium,* and *Penicillium chrysogenum, Alternaria, Phoma, Curvularia, Cladosporium, Eurotium* and *Pitchia burtonii*.

By storing these products in favorable conditions of temperature and relative humidity the intensity of infestation of these fungi increased day by day. Microbial spoilage in bakery products causes large economic losses for both bakery industry and consumer. Fungal spoilage is the main cause of substantial economic losses in packaged bakery products and might also be regarded as sources of mycotoxins involving public health problems. Therefore control of fungal spoilage in the bakery products is extremely important and deleterious effects can be alleviated through integrated approaches. The present findings gain support from the work of Patil and Kudade (2020) [35], Ionnis vageles et al., (2014) [36], Unachukwu and Nwakanma (2015) [37].

V. Conclusions:

Fungi can spoil most types of bakery products including breads. The spoilage can appear in a number of forms and shows visible growth on product surfaces, gas production leading to product damage or pack expansion odor and flavor changes caused by fungal metabolism. Therefore, it is a pity that fungal spoilage of bakery products has received more attention. Sources of fungi in bakeries have been identified and the most important factors governing the fungal spoilage of bakery products established. Mold spoilage is still a major problem limiting the shelf life of many high and intermediate moisture bakery products. Losses due to mold spoilage have been resulting in lost revenue to the baking industries. Therefore, methods to control mold growth and to extend the shelf life of bakery products is of great economic importance to the bakery industries where an increased demand in global consumption exists. Other measures as good hygiene in the bakeries and if

necessary complementary post packaging heat treatments or modified atmosphere packaging is the best alternatives. More investigation is needed in natural preservatives and Map for preservation of these products.

Use of antifungal reagent such as natural agents like clove powder has been recommended to minimize fungal infestations. Lemongrass extract shows the greatest antifungal activity and may be recommended. Cardamom powder also shows the significant activity against the fungi. The synthetic chemicals are hazardous to the human health and therefore use of natural agents is useful to maintain human health. Thus by using above natural agents we can control the fungal spoilage of bakery products.

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