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Study on fungi associated with spoilage of bread

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Abstract

Bread is one of the staple foods in the world and it is recognized as a semi perishable commodity. Usually the spoilage of bread is due to improper storage. The fungi associated with the spoilage of bread were studied. Mold spoilage of bread is due to post processing contamination. Twenty bread samples were collected from different shops in Jaffna. The fungi involved during spoilage were isolated and identified based on the cultural and morphological characteristics using the standard keys available. Initially the molds namely *Mucor* sp and *Rhizopus* sp were found to be the cause of bread spoilage. This was followed by *Aspergillus* sp and *Penicillium* sp. In this study *Rhizopus sp* was found to be the most common fungus during the spoilage of bread.

Keywords: Bread, spoilage, *Mucor* sp, *Rhizopus* sp.

Introduction

Bakery products, like bread has become an important staple food in many countries. Cereals and bakery products serve as a valuable source of nutrients in the diet of many people. They provide most of our food calories. Bakery products provide nutrients such as carbohydrates, proteins, lipids vitamins and minerals. A variety of bakery products are available in the market. Earlier bakery products were considered as a sick man's diet or poor man's diet. It has now become the essential food item for a vast majority of the whole population. Bread is made by mixing flour, salt, yeast and other ingredients which is followed by baking. The basic process involves mixing the above ingredients until the flour is made into dough. The dough is baked into a loaf. The dough is made in such a way that will rise easily and be able to give a bread of good quality to the consumer. Yeast is used in the dough which releases CO_2 and the bread becomes spongy. Earlier airborne yeasts were used in making bread. This was done by keeping the dough exposed to air for sometime before baking. But the technology has improved the bread making to a greater extent in which high energy mixing is involved.

Usually the mold spoilage of bread is due to post processing contamination. Bread loaves fresh out of the oven are free of molds or mold spores due to their thermal inactivation during the baking process (Ponte and Tsen, 1978). Bread becomes contaminated after baking, from the mold spores present in the atmosphere surrounding loaves during cooling, slicing, packaging and storage. Most common source of microbial spoilage is due to mold growth. According to the previous studies (Banwart, 2004) bread molds like *Mucor* and *Rhizopus* are found to grow first during bread spoilage. This is followed by some other fungi like *Aspergillus, Penicillium* and *Fusarium* sp.

Among these *Penicillium* sp is the most common one though *Aspergillus* sp may be of greater significance in tropical countries (Legan, 1993).

Materials and Methods

The 10 loaves of bread used in this study were purchased from different shops in the local market. The samples collected were brought in sterile polythene bags to the laboratory for analysis. These were exposed to the laboratory environment for 7 days. All the glass wares used in this study were sterilized in a hot air oven at 160 ˚C for 2 hrs. The other materials were sterilized by autoclaving at 121 ˚C for 15 mins. Potato Dextrose Agar (PDA) which is a common medium to grow fungi was used in this study. 39g of PDA was dissolved in 100ml distilled water. Then this medium was autoclaved at 121 ˚C for 15 mins. After sterilization it was allowed to cool down to about 50 ˚C. About 20 ml of the medium was poured into each sterilized petri dish. The PDA medium in petri dish was allowed to solidify.1g bread sample was mixed with distilled water and a homogenate was prepared. Dilution plate method was carried out to enumerate the fungi, The working surface was sterilized using ethanol. 1ml was taken from the above homogenate. Serial dilution was done at the recommended dilution rate *i.e* 1:10 (1+9). Dilution was done using saline water. Aliquots are drawn for dilution within one min because fungal spores sediment quickly than bacteria (Beuchat, 1992). Dilutions up to 10^{-4} were carried out. 0.1 ml of the inoculum was added on the surface of the PDA medium and spreaded evenly over the surface using a sterile spreader (bent glass rod). The plates were incubated in an upright position at 30˚C for 4-5days. The same procedure was carried out for all the samples. The fungal count was recorded. The different types of colonies were used as inocula to obtain pure cultures by sub culturing in PDA. A small portion of each sub-cultured colony was cut using a sterile scalpel. It was placed on a sterile glass slide using a sterile forceps. The slide was covered with a cover slip and placed in a petridish. Similar procedure was carried out for other fungal colonies as well..These petridishes were left at 30 ˚C for 5days. The cover slips were taken with forceps and placed on slides containing cotton blue. The excess stain was removed and observed under the microscope. The morphology ie shape, structure of conidia, conidiophores, pigmentation, shape of sporangia, sporangiophores were recorded. The identification was based on the standard keys available.

Results and Discussion

After 5 days of incubation period, the range of fungal count was 7-10 x 10^4 colonies per plate on average. Fungal growth was not observed during the first two days in all the bread samples. But fungal growth was observed in all the samples from the fourth day. The fungal count increased with the days of storage. Table 1 shows the isolated fungi based on the cultural and morphological characteristics.

Table 1 Cultural and morphological characteristics of identified fungi

Fungal Isolate	Cultural Characteristics	Morphological Characteristics
Mucor sp	Large white colonies which turns into black later.	Erect sporangiophores are formed. Sporangiophore swells ate the tip to form sporangia which are globular shaped. Columella is present.
Rhizopus sp	White cottony mycelia, with black dots and covers the entire plate.	Sporangiospores are produced inside a spherical sporangium. Columella is present on the top of the sporagiophore. Root-like rhizoids are found.
Penicillium sp	Fast-growing colonies in green colour with dense conidia	Branched conidiophores with chains of conidia looks like a brush.
Aspergillus sp	Yellow or yellowish green colonies with distinct margin	Conidiophores arise from a foot-cell. Club shaped vesicles at top of the conidiophores. Conidia are found in chains.

The no of colonies increased with days. After 7 days of storage, the highest fungal count was observed in all the samples.

Table 2 Frequency of occurrence of fungi

Day	Fungus	Frequency (%)
1-3	No growth	0
4	*Mucor* sp	70
5	*Rhizopus* sp	80
6	*Aspergillus* sp	20
7	*Penicillium* sp	15

Total no of samples=10

% frequency = (No of occurrence of fungi/Total No of samples) x 100

This study showed that the mold growth increased with the No of days. It depends on the storage conditions. The order of particular fungal growth may be related to the constituents available in the substrate and the physical parameters in which further studies have to be done.

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