



ISOLATION AND CHARACTERIZATION OF FUNGI ASSOCIATED WITH STALED BREAD IN SIDDIPET DIVISION OF TELANGANA STATE

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Abstract

Bread is the most important staple food in the siddipet and it is recognized as a perishable commodity, which is at its best when consumed 'fresh'. Unfortunately, bread remains 'fresh' for only a few hours after it leaves the oven. Isolation and characterization of fungi associated with staled bread in siddipet places was investigated. The aim of this research is to examine the fungi associated with staled bread in siddipet places. The bread sample used for this study was collected from different places of siddipet, the glass wares were sterilized properly in hot air oven at 160°C for two hours, other materials were sterilized by autoclaving at 121°C for 15 minutes. The culture media used for this experiment is Sabouraud Dextrose agar (SDA)

which is known to support the growth of only fungal organisms. The organisms found to be associated with the spoilage of bread were strictly fungal organisms which include; *Mucor spp.* (30%), *Rhizopus spp.* (17%), *Penicillium spp.* (17%), *Fusarium spp.* (3%). However, the percentage distribution of fungal organism isolated from staled bread in different places of siddipet showed that bakery had the highest percentage of 23% followed by Kirana shop (17%) & super market (8%). After analysing the samples, *Aspergillus spp.* was found to be the most occurring fungi in bread.

Keywords

Bread spoilage, Staled bread, *Mucor spp.*, Sabouraud Dextrose Agar (SDA).

Introduction

Bread is the most important staple food in the Western world and it is recognized as a perishable commodity, which is at its best when consumed 'fresh'. Unfortunately, bread remains 'fresh' for only a few hours after it leaves the oven. During storage it is subjected to a number of changes which lead to the loss of its organoleptic freshness. The factors that govern the rate of freshness loss in bread during storage are mainly divided into two groups; those attributed to microbial attack, and those that are result of a series of slow chemical or physical changes which lead to the progressive firming up of the crumb, commonly referred to as 'staling'. Microbiological spoilage of bread the most common source of microbial spoilage of bread is mould growth.

Less common, but still causing problems in warm weather, is the bacterial spoilage condition known as 'rope' caused by growth of *Bacillus* species. Least common of all types of microbial spoilage in bread is that caused by certain types of yeast. The scientific names of fungi that grow on bread are; *Rhizopus nigricans* and *Mucor stolonifera* (Ban wart, 2004). There are minor differences between the two and both are commonly referred to as "Bread mold". These are invariably the first one to "arrive" and germinate on a piece of bread. Later, many others may follow such as *Aspergillus Penicillium* (Hocky,). Yeast is used in the dough of the bread to release CO₂ and makes the bread spongy and fluffy. Bread is one of the oldest prepared foods. Evidence from 30,000 years ago in Europe revealed starch residue on rocks used for pounding plants. It is possible that during this time, starch extract from the roots of plants, such as cattails and ferns, was spread on a flat rock, placed over a fire and cooked into a primitive form of flatbread. Around 10,000 BC, with the dawn of the Neolithic age and the spread of agriculture, grains became the mainstay of making bread. Yeast spores are ubiquitous, including the surface of cereal grains so any dough left to rest will become naturally leavened. There were multiple sources of leavening available for early bread. Airborne yeasts could be harnessed by leaving uncooked dough exposed to air for some time before cooking. Alexander, M. (1999). The Mycoflora of Corn Silage. Journal of Veterinary Medicine, 23 Banwart, G. J. 2004. Basic Food Microbiology.

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Material And Methods

Study Area

The study was carried out in the Government Degree & Pg. College (A) Siddipet.

The material used in this study include; Sabouraud Dextrose agar, Brain Heart Infusion, Aluminum foil, Spirit lamp, Petri dishes, measuring cylinder, Conical flask, Refrigerator, Staining rack,

Incubator, Hot air oven, Weighing balance.

Sterilization Of Material

Media prepared was sterilized in an autoclave at 121°C for 15 minutes, all glass wares sterilized in a hot air oven at 160°C for 1 hour. Before the glass wares were sterilized in hot air oven they meticulously washed with detergent, rinsed in distilled water.

The methods used in this experiments were carried out according to standards recommended by the following researchers (Alexander (1999), Harrigan (1988), Dubey and Maheshawi (2004).

SAMPLE SIZE The sample size that was used in this work was calculated using Lesile Kish formular (kish., 1995):

$$N = Z^2 \times P (1-P)/d^2$$

Where;

N = Minimum sample size

D = desired level of significance (0.5) Z = confidence interval (1.96)

P = prevalence rate 6%

30 samples where used

Collection Of Samples

The 30 rolls of bread used for this study were purchased from different Bakery's, supermarkets & Kirana stores. The samples collected were transported in a sterile polyethylene bag to the laboratory for analysis.

Media Preparation:

The culture media used for this experiment is the sabouraud dextrose agar (SDA) which is known to support the growth of only fungi organisms and the Brian heart infusion agar. The media was prepared according to the manufacturer's directions.

All the glass wares used for this study were sterilized properly in a hot air oven at 160°C for an hour. Other materials were sterilized by autoclaving at 121°C for 15minutes.

32.5g salt was weighed out with a weighing balance for 500mls of water. The two mixtures were mixed together and sealed with aluminum foil and autoclaved at 121°C for 15minutes.



Figure : 1 Preparation Of Media

Isolation Of Fungi:

The samples were kept in different Siddipet division place for 5 days. It was then transported to the laboratory in a black polyethylene bag.

The total of 30 rolls of bread samples were used in this research. Sterile Petri dishes were aligned and Sabouraud Dextrose Agar (DSA) media already prepared were poured into the Petri dishes, they were allowed to gel. The plates dried in inverted position. 1g of each of the sample was matched and spread on the media plate. It was sealed with paper tape and then incubated for 5 days at 27°C for colony formation. The count was determined by counting the corresponding colonies that were observed. Spread plating techniques was used for discrete colonies. The count was recorded in colony forming unit per ml (CFU/ml).



Figure 2& 3 : Isolation Of The Media

Results

After incubation period, the total fungal count of bread samples over a storage period of 5 days is shown below;

There was no fungal count on the first two days of study for the thirty (30) samples used. On the third day of the study, nine (9) out of the sixty (30) samples had scant fungal count. However, all the samples showed positive fungal growth from the fourth day till the fifth day.

Table 1 shows the percentage occurrence of positive and negative growth of fungal organism isolated from staled bread collected from Siddipet division with a hundred percent growth.

Table 2 shows the percentage distribution of fungal isolated from staled bread collected in Siddipet division. *Mucor spp*, *Fusarium spp*, *Aspergillus spp*, *Rhizopus spp*, and *Penicillium spp*.

Table 4.3 shows the percentage distribution of fungal isolated from staled bread collected within different Siddipet division. *Aspergillus spp* has the highest percentage of 33 followed by *Mucor spp*. (30%), *Rhizopus spp*. (17%), *Penicillium spp*. (17%), *Fusarium spp*. (3%).

Table 1 :Percentage Occurrence Of Positive And Negative Fungal Organism Isolated From Bread Different Places Of Siddipet

S no	location	No of samples	No of positive %	No of negative%	p. value
1	bakery	10	10(100)	0(0)	0.7
2	Super market	10	10(100)	0(0)	0.5
3	Kirana shop	10	10(100)	0(0)	0.31
	Total	30	30(100)	0(0)	

KEY: NO = NUMBER

 $P < 0.03$ **TABLE 2: Percentage Distribution Of Fungi Isolated From Bread In Different Places Of Siddipet.**

Fungal isolates	Number	Percentage	P.value
Fusarium spp	2	3	0.21
Rhizopus spp	10	16	0.16
Penicillium spp	11	17	0.00
Mucor spp	16	30	0.88
Aspergillus spp	21	34	.487
TOTAL	60	100	

*P < 0.05



Figure 4: Distribution Of Fungal Isolates In Siddipet

TABLE 3: The Percentage Of Distribution Of Fungi Isolates Within Different Location Of Siddipet Division

Fungal Isolate	bakery	Super market	Kirana shop
Rhizopus spp	2(16)	3(33)	0(0)
Aspergillus spp	3(23)	3(60)	5(36)
Mucor spp	4(31)	2(22)	18(30)
Penicillium spp	2(15)	10(17)	2(20)
Fusarium spp	3(15)	0(0)	0(0)
Total	14(22)	18(8)	25(16)

*=P < 0.05



Figure 5: Fungi Moulds On PDA

Discussion

The study was done to isolate the fungi associated in staled bread in different places of siddipet. A total number of 30 samples were collected during the course of this research. After incubation period, the total fungal count of bread samples over a storage period of 5 days is shown in Table 4.1. There was no fungal count on the first two days of study for the sixty (30) samples used. On the third day of the study, twenty-nine (9) out of the sixty (30) samples had scanty fungal count. However, all the samples showed positive fungal growth from the fourth day till the fifth day with similar findings by Gide *et al.*, (2016). There was no significant difference ie $p < 0.05$. The observations show 100% growth of fungal organism.

The fungal load (count) increased progressing as the period of storage increased. The fifth day therefore showed the highest fungal count for all examined samples. The fungal organism isolated from this study includes; *Mucor spp*, *Fusarium spp*, *Aspergillus spp*, *Rhizopus spp*, and *Penicillium spp*. Of which *Aspergillus spp* has the highest percentage of 33 followed by *Mucor spp*. (30%), *Rhizopus spp*. (17%), *Penicillium spp*. (17%), *Fusarium spp*. (3%) This is in line with a similar work by Gouse *et al.*, (2015) their findings had a total number of 100% fungal isolates belonging to 5 genera namely *Mucor spp*, *Fusarium spp*, *Aspergillus spp*, *Rhizopus spp*, and *Penicillium spp*.

From this study, the percentage distribution of fungal organism isolated from staled bread used within different places of siddipet showed that bakery had the highest percentage of 23 followed by Kirana shop (17%) & supermarket (8%). (Table 3), this is in agreement with a similar finding by Gouse *et al.*, (2015) whose observation showed that siddipet had the highest frequency percentage. There was significant difference

$p < 0.05$

Conclusion

The findings of the study revealed that staled bread in different places of siddipet were contaminated with various fungi. This indicates inadequate and poor maintenance. The presence of the fungi is an indication of improper cleaning of the shops. These fungi could result to respiratory tract infections e.g., sinusitis, aspergillosis etc. of which these breads can serve as medium to their transmission to individuals. These diseases can easily affect immune compromised individuals.

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Conflicts Of Interest

The authors declare no conflict of interest.

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