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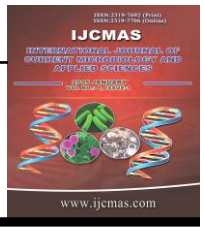

Original Research Article

The fungi associated with the spoilage of bread in Enugu state

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A B S T R A C T

Fungi associated with the spoilage of bread in Enugu was investigated. The 30 bread samples used for this study were collected from different vendors in Enugu and exposed for 7days at different locations in the University environment and observed daily for spoilage. The glass wares used for this study were sterilized properly in hot air oven at 160^0C for two hours, other materials were sterilized by autoclaving at 121^0C for 15minutes. The culture media used for this experiment is the Sabouraud's 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; *Rhizopus spp, Aspergillus spp, Mucor spp, Penicillium spp, and Fusarium spp*. These isolates were identified bacteriologically by their cultural morphological characteristics. After analyzing the samples, *Rhizopus* was found to be the most occurring fungi in bread.

Keywords

Microorganism, Bread, *Penicillum* spp, *Rhizopus* spp, Enugu state

Introduction

The scientific names of fungi that grow on bread are; *Rhizopus nigricans* and *Mucor stolonifer* (Banwart, 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* and *Penicillium* (Hocky, 2008). Yeast is used in the dough of the bread to release CO_2 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. Pliny the Elder reported that the Gaul's and Iberians used the foam skimmed from beer used a paste composed of grape juice and flour that was allowed to begin fermenting, or wheat bran steeped in wine, as a source for yeast.

The most common source of leavening was to retain a piece of dough from the previous day to use as a form of sourdough starter (Seiler, 2000). In 1961, the Chorleywood bread process was developed, which used the intense mechanical working of dough to dramatically reduce the fermentation period and the time taken to produce a loaf. The process, whose high energy mixing allows for the use of lower protein grain, is now widely used around the world in large factories. As a result, bread can be produced very quickly at low costs to the manufacturer and the consumer.

Formulation and bread making

Professional baker recipes use a notation called baker's percentages. The amount of flour is usually 100%, and the amounts of the other ingredients are expressed as a percentage of that amount by weight. Measurement by weight is more accurate and consistent than measurement by volume, particularly for dry ingredients. The proportion of water to flour is the most important measurement in a bread recipe, as it affects texture and crumbs the most.

Hard US wheat flours absorbs about 62% water, while softer wheat flours absorb about 56%. Common table breads made from this dough's result in a fine textured, light bread. Most artisan bread formulas contain anywhere from 60 to 75% water. In yeast breads, the higher the water percentages result in more Co2 bubbles and a coarser bread crumb. One pound (450g) of flour will yield a standard loaf of bread or two French loaves. Calcium propionate is commonly added by commercial bakeries to tetrad the growth of molds (Seiler, 1994).

Flour

Flour is a product made from grain that has been ground to a powdery consistency. Flour provides the primary structure to the final baked bread. While wheat flour is most commonly used for breads, flours made from rye, barley, maize and other grains are also commonly available. Each of these grains provides the starch and protein needed to form bred. The protein content of the flour is the best indicator of the quality of the bread dough and the finished bread. While bread can be made from all-purpose wheat flour, a especially bread flour, containing more protein (12 – 14%), is recommended for high quality bread. If one uses a flour with a lower protein content (9 - 11%) to produce bread, a shorter mixing time will be required to develop gluten strength properly.

An extended mixing time leads to oxidation of the dough, which gives the finished product a whiter crumb, instead of the cream color preferred by most artisan bakers (Seiler, 2000). Wheat flour, in addition to its starch, contains three water- soluble protein groups (albumin, globulin, and proteases) and two water- soluble protein groups (glutenin and gliadins).

When flour is mixed with water, the water-soluble proteins dissolve, leaving the glutenin and gliadin to form the structure of the resulting bread. When relatively dry dough is worked by kneading, or wet dough is allowed to rise for a long time, the glutenin forms strands of long, thin,

chainlike molecules, while the shorter gliadin forms bridges between the strands of glutenin. The resulting network of strands produced by those two proteins is known as gluten. Gluten development improves if the dough is allowed to autolyse.

Penicillium spp

Penicillium chrysogenum is a fungus, common in temperature and subtropical regions and can be found on salted food products, but it is mostly found in indoor environments, especially in damp or water-damaged buildings. It is the source of several B-lactam antibiotics, most significantly penicillin. Other secondary metabolites of *P. chrysogenum* include various penicillin, *Roquefortine, C. melsagrin*, *Chrysogenum,* and *Xanthocillins*,

However, *P. chrysogenum* cannot be identified based on color alone. Observations of morphology and microscopic features are needed to confirm its identity and DNA sequencing is essential to distinguish it from closely related species such as *Penicillium rubens*. *P. chrysogenum* has been used industrially to produce penicillin and xanthocillin x, to treat pulp mill waste, and to produce the enzymes polyamine oxidase, phosphor-gluconate dehydrogenate, and glucose oxidase (Jay, 1998).

Mucor spp

Mucor spp is a filamentous fungus found in the soil, digestive system, decayed fruits, vegetables and old bread. *Mucor spp* may cause infection in man, frogs, amphibian's cattle and swine. Most of the *mucor spp* are unable to grow at 37^0C and the strains isolated from human infections are usually one of the few Thom tolerant mucor spp (Ronald, 1994).

Materials and Methods

The 30 rolls of bread used for this study were purchased from different vendors in Enugu, Nigeria. The samples collected were transported in a sterile polyethylene bag to the laboratory for analysis. The culture media used for this experiment is the sabouraud dextrose agar (SDA) which is known to support the growth of only fungi organisms. 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 160^0C for an hour. Other materials were sterilized by autoclaving at 121^0C for 15minutes.

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). 8.5g salt was weighed out with triple beam balance for 100mls of water. The two mixtures were mixed together and sealed with aluminum foil and autoclaved at 121^0C for 15minutes. Three test tubes containing 9ml of sterile normal saline were on a rack on the working bench. 1gram of each sample was dissolved into the first test tubes and mixed thoroughly. 1ml of the sample was pipette aseptically into the first test tube and mixed, this was repeated serially to the last tube (10^{-3}).

Then 1ml from the last tube was discarded. The table was cleaned with 70% ethanol using cotton wool, the samples bought were labeled and placed accordingly on the table. The samples were kept on the table with the Bunsen burner on to keep the working place sterile and free from unwanted organisms. 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, sealed with paper tape and then incubated for 1 week at 37^0C for colony formation. The count was determined by counting the corresponding colonies that were observed after the 1ml of the serially diluted samples. Spread plating techniques was used for discrete colonies within the 1ml inoculums. The count was recorded in colony forming unit per ml (CFU)ml).

A small portion of each sub-cultured colony was cut out using a sterile dissecting blade. It was then picked up with a sterile forceps and placed on a new, sterile glass slide; the slide was then covered with a cover slip, kept in a slant in new Petri dishes. The Petri dishes were left on the bench for 5 days. The cover slips were carefully picked respectively with forceps and dropped on the slides containing lacto-phenol.

The slide preparation was carefully covered with cover slips with the exclusion of air bubbles. Blotting paper was used to remove excess stain coming through the edge of the cover slip. Slides of each colony were made and observed under the low power objective (x10) and high power objective (x40) lens of the compound microscope.

The spore type, surface texture, pigmentation and the pigmentation of the reverse side of the plate; the colonies formed were also recorded. If there is no sign of mold growth, the box will be ticked 'X'. If mold growth is found, the box is ticked 'Y'.

Results and Discussion

After incubation period, the total fungal count of bread samples over a storage period of 7 days are shown in Table 1. It had a fungal range of 6-8 x 10^3 cfu. 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, eighteen (18) out of the thirty (30) samples had scant fungal count. However, all the samples showed positive fungal growth from the fourth day till the seventh day.

Plate.1 Serial Dilution Process

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, therefore to calculate (% frequency) = $\frac{x}{y} \times \frac{100}{1}$

The fungal load (count) increased progressing as the period of storage increased. The seventh day therefore showed the highest fungal count for all examined samples. The isolated organisms are; *Mucor spp, Fusarium spp, Aspergillus spp, Rhizopus spp, and Penicillium spp.* Table 3. shows the Identification of the isolate based on cultural characteristics, colony morphology (cell size, shape, pigmentation and arrangement). Result from this experiment indicates the environment as the independent variable in which the bread slice is kept. While the dependent variable is the growth of bread mold. The constants (control variables) are the room temperature, the age of the bread and the handling of the bread. (Table 2).

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