E-ISSN: 1116 - 3089 P-ISSN: 3026-9784
MPRI-JOMDR | INTERNATIONAL JOURNAL OF ORGANIC & MEDICINAL DEV. RESEARCH, VOL. 14 NO. 3 OCTOBER, 2026

FUNGAL STUDY OF SPOILED BREAD HAWKED IN OKO

BALA USMAN ABDULLAHI

Department of Microbiology, Federal Polytechnic Oko, Anambra State.
Corresponding Author: balausman605@gmail.com
DOI: https://doi.org/10.70382/mejomdr.v14i3.030

Abstract

This study aimed in fungal assessment of spoiled bread hawked in Oko. The analysis was carried out with five different bread samples which were randomly purchased from different vendors at Oko and was aseptically sent to microbiology laboratory for fungal analysis, 1 gram of each of the sample were serially diluted and inoculated using the spread plate method after which it was incubated at 72-96 hours at 24°C. The viable fungi colony count were 2 x 10^{-1}cfu/g, 24.4 x 10^{-1} cfu/g, 13.4 x 10^{-1} cfu/g, 29.6 x 10^{-1} cfu/g and 26.4 x 10^{-1} cfu/g. The finding of this study showed that the colony forming unit did not exceed the National Agency for Food, Drug, Administration and Control (NAFDAC) standard of 10^3cfu/g. After the fungi analysis the probable organism gotten are *Neurospora spp., Rizhopus spp, Conidiobolus spp, Aspergillus spp* and *Penicillum spp. Neurospora spp.* was found to be the most occurring fungi in bread samples. It is recommended that measures as good hygiene in the bakeries and if necessary complementary post packaging heat treatments or modified atmosphere packaging is the best alternative.

Key words: fungal study, spoiled bread, Rizhopus spp, Aspergillus spp, penicillium spp.

Introduction

Bread is a staple food prepared from dough of flour and water usually by baking. Throughout recorded history it has been popular around the world and is one of the oldest artificial foods, having been of importance since the dawn of agriculture (Unachukwu and Nwakanma, 2015). There are many combinations and proportions of types of flour and other ingredients, and also of different traditional recipes and modes of preparation of bread. As a result, there are wide varieties of types, shapes, sizes and textures of bread in various regions. Bread may be leavened by one of many different processes, ranging from reliance or naturally occurring microbes (so called "sourdough" recipes) to addition of chemicals or industrially produced yeast to high pressure artificial aeration method during preparation or baking (Banwart, 2018).

However, bread becomes contaminated after baking by the mould spores present in the atmosphere surrounding the loaves during cooling, slicing, packaging and storage. Most common

EDITOR-IN-CHIEF
Prof. A.S. Hossein [Saskatchewan]

source of microbial spoilage is due to mould growth which occurs when mould spores land on the bread surface and consumes nutrients and moisture in the bread resulting in growth (Legan, 2015). Spoilage by microbial growth causes economic loss for both the manufacturers and consumers. Members of the genus *Bacillus* bring about bacterial spoilage of bread known as ''rope''. Ropiness is the most important spoilage of bread after mouldiness which occurs particularly in summer when the climate conditions favour growth of bacteria (Young *et al.,* 2017). Commercially produced and properly handled bread generally lacks sufficient amount of moisture to slow growth of any micro-organisms except moulds. As normal cooking temperature destroys fungal spores, post-process contamination from air-borne spores and contact with contaminated surfaces must be prevented. Losses of bakery products due to mould spoilage vary between 1-5% depending on seasons, type of products and method of processing (Banwart, 2018). Effects of contaminated bread in the society can lead to an epidemic of gastro-intestinal disorder whereby that population that consume the contaminated bread become affected thereby developing stomach upset. According to the previous studies of Banwart (2015), bread mould like *Mucor* and *Rhizopus* are found to grow first during bread spoilage. This is followed by other fungi like *Aspergillus, Penicillum* and *Fusarium sp.* (Hocking, 2018). Among these, *Penicillum sp.* is the most common one.

The presence of fungal species such as *Neurospora spp., Rhizopus spp., Conidiobulus spp., Aspergillus spp.,* and *Penicillium spp*. in bread can be attributed to high moisture levels. Warm temperatures can promote faster fungal proliferation. Fungal spores can be present in flour, grains, or other ingredients used in bread-making (Edwards, 2019). Improperly cleaned tools and surfaces can harbor spores. Contaminated packaging materials can introduce fungi in bread. Fungi often thrive in slightly acidic to neutral pH levels, which may be present in certain types of bread (Hamelman, 2015). Contact with contaminated surfaces, other food items, or even during handling can transfer fungal spores in bread. Insects can introduce or spread fungal spores in bread. Understanding and controlling these factors can help mitigate fungal contamination in bread and other baked goods (Kourkouta, 2017).

Collection of Samples

The samples of spoiled breads were bought from different hawkers within Oko, Orumba North Local Government Area of Anambra State. The samples were forwarded to the Laboratory for its microbiological investigation.

Sterilization

The glass was washed with water and detergent, rinsed with clean water and sterilized by hot air oven, wrapped in aluminium foil or placed in a metal container with a tight-fitting, closed lid. Depending on the temperature selected, the total cycle time (preheating, sterilization time and cool down) was ranged from about 2.5 hours at 170qC to more than 8 hours at 121qC.

Preparation of Sabouraud Dextrose Agar:

15.6g of Sabouraud Dextrose Agar (SDA) was weighed and dissolved in 240ml of distilled water. The solution was poured into conical flask and the mouth was plugged with cotton wool and was covered with aluminum foil and was allowed to cool to about 45°C. The molten Sabouraud Dextrose Agar (SDA) was poured into well labelled sterile petri-dishes and was allowed to solidify.

EDITOR-IN-CHIEF
Prof. A.S. Hossein [Saskatchewan]

Inoculum and Preparation

1g of each sample was weighed with electronic weighing balance and was added to 9ml of distilled water on separate test tube which was labelled as the stock cultures. 1ml of each sample was drawn from each stock culture and transferred to the next test tube containing 9ml of distilled water, while into each of the 5 test tube, 9ml of distilled water was added and serial dilution was carried out for each sample using separate syring for each dilution.

Identification of Isolates

Identification of isolates was based on the cultural, morphological and identification characteristics.

Cultural Test

Cultural test of the colonies on the agar plates was observed for elevation, nature of colony growth, colour of colonies and surface appearance.

Identification of FungiLactophenol Cotton Blue Test

Lactophenol cotton blue stain are used to visualize certain fungal structures that are significant for their identification. These include the hyphae, spores and germinal tube. Lactophenol cotton blue components are phenol which kill any living organism; lactic acid preserves the fungal structures and cotton blue stains the chitin in the fungal cell wall. This test was carried out by placing a drop of lactophenol cotton blue staining solution on a clean slide. The fungi culture was mixed with the staining solution. A coverslip was placed on top and was heated gently. The cover slip was pressed gently to remove any bubbles. The slide was observed under oil immersion microscope. Fungal elements are stained intensely blue.

Microscopic and Macroscopic identification of fungi isolation

The microscopic and macroscopic identification of fungi isolated from different spoiled bread samples are presented in table 4.1 The fungi isolated were *Neurospora spp, Rihizopus spp, Conidiobolus spp, Aspergillus spp* and *Penicillum spp.* The table shows the result of the total fungal load obtained from the different spoiled bread hawked in Oko, Anambra State.

Table 1: Macroscopic and macroscopic identification of fungi isolated

Sample	Macrospcic characteristics	Microscopic characteristics	Names of organisms
A	Colour was initially white and later light yellow its odor was mild and earthy	Hyphae walls were thin and smooth pleurocystidia and Cheilocystidia were seen	*Neurospora spp*
B	Colour was initially white and later yellow with a pinkish tint. Odor was musty	Hyphae wall was thin and smooth, oval in shape, chlamysospores were seen	*Rhizopus spp*
C	Colonies were white, grayish in colour. Edge were irregular	Hyphae wall were thin and smooth condilphores were short	*Conidiobolus spp*

EDITOR-IN-CHIEF
Prof. A.S. Hossein [Saskatchewan]

Sample	Macrospcic characteristics	Microscopic characteristics	Names of organisms
D	Colonies were initially white and later becoming dark	Soma is filamentous with septate hyphae, simple and thick walled	*Aspergillus spp*
E	Colonies were white in colour with irregular edge	Hypae wall were thin and smooth septa were present, conidiophores were tree like branched	*Penicillum spp*

Key:
A = Eke Oko
B = Oko Roundabout
C = Total Market Oko
D = Tipper Road Oko
E = Main Gate Oko

Fungal Loads of Spoiled Bread

Table 2. Total Viable Fungal (cfu/g) of Spoiled Bread

Samples	No of colonies	Dilution factors	Fungi count cfu/g
A	320	10^{-1}	32.0×10^{-1}
B	244	10^{-1}	24.4×10^{-1}
C	134	10^{-1}	13.4×10^{-1}
D	296	10^{-1}	29.6×10^{-1}
E	264	10^{-1}	26.4×10^{-1}
Control	-	-	-

Key:
A = Eke Oko
B = Oko Roundabout
C = Total Market Oko
D = Tipper Road Oko
E = Main Gate Oko

DISCUSSION

Fungal Investigation of spoiled bread hawked in Oko, Anambra State was investigated in this study. The analysis was carried out with five different spoiled bread which were randomly purchased from different vendors and was aseptically sent to microbiology laboratory for fungal analysis, 1 gram of each was serially diluted and inoculated using the spread plate method after which it was incubated at 72-96 hours at 24°C.

EDITOR-IN-CHIEF
Prof. A.S. Hossein [Saskatchewan]

The viable fungi colony count were 3.2 x 10^{-1}cfu/g, 24.4 x 10^{-1}cfu/g, 13.4 x 10^{-1}cfu/g, 29.6x10^{-1}cfu/g and 26.4x10^{-1}cfu/g. The Macroscopic and Microscopic identification was carried out, the probable microorganisms include *Neurospora spp., Rihizopus spp, Conidiobolus spp, Aspergillus spp* and *Penicillum spp* were predominant fungi as it was isolated in about five of the spoiled bread. This observation was in accordance to Unachukwu and Nwakanma (2015) who worked on the fungi associated with the spoilage of bread in Enugu state. Their results showed that 124% of samples were contaminated with species *Rhizopus spp*, *Aspergillus spp*, *Mucor spp*, *Penicillium spp* and *Fusarium spp*. It had a fungal range of 17.8-29.4 x 10^3 cfu. The organisms found to be associated with the spoilage of bread were strictly fungal organisms which include; *Rhizopus spp* (24.3.0 x 10^3), *Aspergillus spp* (29.4 x 10^3), *Mucor spp* (20.3 x 10^3), *Penicillium spp* (26.3 x 10^3), and *Fusarium spp* (17.8 x 10^3).The study is also in accordance to the study of Ponte
and Tsen (2022) who worked on Isolation and Identification of Fungi Associated with the Spoilage of Some Selected Bread Sold within Anyigba Metropolis. In their work, fungi isolated were *Aspergillus flavus* (28.6 x 10^2), *Aspergillus fumigatus* (27.5 x 10^2), *Aspergillus niger* (29.6 x 10^2), *Penicillium sp* (25.6 x 10^2). *Aspergillus niger* (29.6 x 10^2) had the highest frequency of occurrence while *Penicillium sp* (25.6 x 10^2) had the least level of occurrence.

The presence of fungal species such as *Neurospora spp., Rhizopus spp., Conidiobulus spp., Aspergillus spp.,* and *Penicillium spp*. in bread can be attributed to high moisture levels. Warm temperatures can promote faster fungal proliferation. Fungal spores can be present in flour, grains, or other ingredients used in bread-making (Edwards, 2019). Improperly cleaned tools and surfaces can harbor spores. Contaminated packaging materials can introduce fungi in bread. Fungi often thrive in slightly acidic to neutral pH levels, which may be present in certain types of bread (Hamelman, 2015). Contact with contaminated surfaces, other food items, or even during handling can transfer fungal spores in bread. Insects can introduce or spread fungal spores in bread. Understanding and controlling these factors can help mitigate fungal contamination in bread and other baked goods (Kourkouta, 2017).

CONCLUSION

From the data and results obtained in this research, bread was shown to be seriously contaminated after 7 days and is unfit for human consumption as a result of the growth of these toxic producing different fungal species due to improper storage facilities and which would cause toxin which can cause serious health problem to human beings and even animals when consumed.

References

Bailey, C. P. and Holy, A. V., (2021). *Bacillus* spore Contamination Associated with Commercial Bread Manufacture. *Food Microbiology*, 10: 287-294.

Banwart, G.J., (2015). *Basic Food Microbiology*. Westport: AVI Publication.

Banwart, M. (2018). Isolation and identification of fungal isolate from bread in Bori. *Journal of Food Science and Technology*. 2(3):98-190.

Boskov, H.H. Andreasen, M. F., Nielsen, M. M., Melchior, Larsen, L. Bach, Knudsen K. E., Meyer, A.S. Christensen, L.P. and Hansen, A. (2022). "Changes in Dietary Fibre, Phenolic Acids and Activity of Endogenous Enzymes During Rye Bread-making". *European Food Research and Technology*. 214:33-38.

Desrosier, N. W. (2016). *The Technology of Food Preservation*. Westport AVI Publ.

EDITOR-IN-CHIEF
Prof. A.S. Hossein [Saskatchewan]

Edwards, W. P. (2019). Hydration Ratio for Breads. Westport AVI publ. Inc., Publication.

Finley, S., John H. and Phillips, R. O. (2017). Protein Quality and the Effects of Processing. New York: M. Dekker. P.2.

Gelinas, P., Mckinnon, T. and Carole, M., (2016).''Effect of Wheat Variety, Farming Site and Bread-baking on Total Phenolics''. *International Journal of Food Science and Technology*. 41 (3): 329 - 334.

Hamelman, J. (2015). *Bread: A bakers book of techniques and recipes.* New York: John Wiley.

Hocking, C. S. (2018). Sorbate Spray Application for Protection of Yeast-raised Products. *Baker's Digest*., 52: 47-50.

Hofmann, T., Lindenmeier, M. and Somoza, V. (2015). "Pronyl-Lysine-A Novel Protein Modification in Bread Crust Melanoidins Showing *In Vitro* Antioxidative and Phase I/II Enzyme modulating Activity". *Annals of the New York Academy of Science*. 8095):117-125.

Kansas, W. (2022). 10 Surprising Uses of for a Slice of Bread. Bread: Beyond the Traditional Slice. Pp. 1-8.
Keta, J.N., Aliero, A., Suberu, H.A. and Shehu, K. (2019). Fungi Species Associated with Bread Spoilage in Birnin Kebbi Town, Kebbi State, Nigeria. *Savanna Journal of Basic and Applied Sciences*. 1(1): 136-139

Knight, R. A. and Menlove, E. M. (2016). Effect of the Bread Baking Process on Destruction of Certain Mould Spores. *Journal of the Science of Food and Agriculture*, 10:653-660.
Kourkouta, L. (2017). Bread: Introduction, History and health Benefits. Pp. 1-7.
Legan, J. D. and Vosey P. A. (2017). Yeast Spoilage of Bakery Products and Ingredients. *Journal of Applied Bacteriology*, 70:361-371.

Legan, J.D. (2015). Mould Spoilage of Bread: Mould Spoilage of Bread: The Problem and Some Solutions. *International Biodeterioration and Biodegradation*, 32:33-53.

Malkki, Y. and Rauha, O. (2020). Mould Inhibition by aerosols. *Baker's Digest*., 54:47-50.

McGee, H. (2015). On Food and Cooking Scribner. P.517.

Narins, E. (2022). 21 Types of Bread you May Encounter If You Count Yourself Among Bread's Biggest Fans. Pp. 1-15.
Nirmala, R., Pathmanathan, S. and Selvaratnam, S. (2016). Study on fungi associated with spoilage of bread. *Int. J. Adv. Res. Biol. Sci*. 3(4): 165-167.

O'Connor, A. (2022). An Overview of the Role of Bread in the UK diet. *Nutrition Bulletin* 37:193-212.

O'Connor, A. (2022). Bread Consumption in the UK: What are the Main Attitudinal Factors Affecting Current Intake and its Place in a Healthy Diet? *Nutrition Bulletin* 37:30-36.

Smith, J. P. (2015). Bakery Products. In: Principles and Application of Modified Atmosphere Packaging of Food (Ed. R. T. Parry). Glassgow, UK. Blackies Academic and Professional. Pp. 134-169.

Sofos, J. N. and Busta, F. (2018). Antimicrobial activity of sorbate. *Journal of Food Protection*, 44:614-621.

Strandas, C., Kamal-Eldin, A., Andersson, R. and Aman, P. (2018). ''Phenolic Glucosidesin Bread Containing Flaxseeds''. *Food Chemistry*. 110 (4):997.
Muinde, O.K. and Kuria, E. (2005). Hygienic and sanitary practices of vendors of street foods in Nairobi, Kenya. 5 (1): 1-13.
Unachukwu and Nwakanma (2015). Fungi Associated with the Spoilage of Bread in Enugu State. *International Journal of Current Microbiology and Applied Sciences*. 4 (6) pp. 989-995.

Vaux, P. and Bert, N. (2020). ''Dialect Survey: What Do You Call The End Loaf of Bread? Pp.90.

Voysey, P. A. and Hommond J. C. (2023). Reduced Additive Bread Making Tech. In: Smith J. (Ed). *Tech of Reduced Additive Foods.* Blackie London, Academic and Professional.

Winkler, V. and Sarah, G. (2019). ''Discovery Health ''Is Eating Bread Crust Really Good for You?'' ''. Health.howstuffworks.com.

Young, P., Linda, C. and Stanley, P. (2017). Technology of Bread Making. Berlin: Springer. P.54.

EDITOR-IN-CHIEF
Prof. A.S. Hossein [Saskatchewan]