IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 21, Issue 7 Ser.1 (July. 2022), PP 40-43
www.iosrjournals.org

Isolation and Identification of Fungi Associated With the Spoilage of Some Selected Bread Sold Within Anyigba Metropolis

Azeez Zainab[1], Okoliko Peace[1], Bello Kizito[1*], Zakari David[1] Olaitan Catherine[1], Audu Nasir Opisa [2]

[1]*Department of Microbiology, Kogi State University, Anyigba, Kogi State.*
[2]*Department of Biological Science, Federal University Lokoja, Kogi State, audunasiriopisa@gmail.com*

Abstract
Bread is one of the most consumed staple foods in most countries and cultures. It is a good source of nutrient such as micronutrients and macronutrients that are essential for human health. This study was carried out to isolate and identify fungi that is associated with spoilage of bread sold in Anyigba metropolis. A total number of four (4) samples were randomly selected and analysed (Anyigba bread, Centenary bread, Yale bread and Ostrich bread). Identification of the fungal isolates was done macroscopically and microscopically. The physicochemical properties (moisture content and pH) and proximate properties (Ash content, crude fat, crude protein and carbohydrate content) of the bread samples were determined and recorded. The fungi isolated were Aspergillus flavus, Aspergillus fumigatus, Aspergillus Niger and Penicillium sp. Aspergillus niger had the highest frequency of occurrence while Penicillium sp. had the least level of occurrence. Therefore, the study 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.

Keyword: *Bread spoilage, Anyigba, Mold and Fungi.*

Date of Submission: 28-06-2022 Date of Acceptance: 09-07-2022

I. Introduction

Baked food such as bread is one of the most consumed staple foods in the world. It is a good source of nutrient such as micronutrients and macronutrients that are essential for human health (Potter and Hotchkiss, 2006). Bread is subjected to various spoilage problems which are physical, chemical and or microbial. Bread spoilage is mostly caused by the action of microbes. Various mold involved in spoilage of bread include *Rhizopus, Mucor, Penicillium, Eurotium, Aspergillus and Monilla* (Saranraj and Geetha, 2012). Mold growth in bread can be reduced by a range of techniques which includes; proper hygiene within the bakery to reduce the opportunities for mold spores from gaining access to the product, use of preservatives which reduces or prevent the growth of molds (Banwart, 2004).

Food can be contaminated as a result of spoilage by pathogenic organism. This can lead to an endemic of gastrointestinal disorder among the consumers. The method of standardized Hazards Analysis Critical Control Point (HACCP) is long considered as a choice for ensuring safety of foods (Jay and De Boser, 1999). Hazard analysis critical control point involves identifying places in the production processess where hazard could occur. i.e. critical control point (CCP) and putting monitoring procedures in place to prevent these hazards occurring. Even with this system in place samples still need to be tested for the presence of microorganism (Jay and De Boser, 1999).

The growth of mold in bread is a substrate-saprophyte relationship with the bread providing nourishment and the mold surviving on it as long as favourable environmental conditions prevail. Mold growth in bread is also favored by their ability to synthesize proteolytic and amylolytic enzymes (Baur, 2001). Furthermore, moisture condensation on a bread surface due to packaging while the product is not completely cool, may be conducive to mold growth (Marin *et al.*, 2002).

A major cause of growth of mold in bread is long storage, resulting in the growth of molds which may produce toxins which lead to food poisoning when such foods are consumed (Vytrasova *et al.*, 2002). There is little documented information on the fungal spoilage of bread within Anyigba metropolis, this study is aimed at the isolate and identify the fungi associated with spoilage of bread in Anyigba, Kogi state, Nigeria.

II. Material And Methods

Sample Collection and Processing

Bread Samples were collected into sterile plastic bags from different bakery point of sale within Anyigba metropolis and was taken to the microbiology laboratory where they were kept and monitored daily until spoilage occurred. Portions of each of the spoilt bread were carefully cut with sterile scalpels and one gram each was enriched in sterile sabouraud dextrose broth for twenty-four hours (24 hours).

Proximate analysis of the bread

The Moisture content, pH, Ash content, Crude fat and crude protein content and carborydrate content of the bread was determined following standard procedure as described by Cheesbrough, 2008.

Mold isolation

One milliliter (1ml) of each of the enriched samples were serially-diluted and 0.1ml of the dilution (10^3) was used to inoculate duplicate plates of already prepared sterile sabouraud dextrose agar (SDA) containing 0.05mg/ml chlorophenicol to inhibit bacterial growth. The sabouraud dextrose agar (SDA) was prepared according to the manufacturer's instructions. The media was autoclaved for 121°C for 15mins. The spread plate technique was used for the inoculation. The inoculated plates were incubated at room temperature (22-25^{o}c) for 72 hours after which the fungal colonies that developed were counted, purified by repeated sub culturing and were stored in sabouraud dextrose agar (SDA) slants for identification.

Identification of the Isolates

The isolates were identified macroscopically and microscopically. The colony color, texture and size were observed while the microscopic examination was done using lactophenol blue stain. A drop of the stain was placed on a clean grease-free slide. A small portion of the fungal culture was emulsified on the slide and covered with a coverslip, avoiding bubbles. The slide was thereafter viewed under the microscope. A slide culture of the fungal isolate was also prepared and examined under the microscope. The cultural features observed were compared with those contained in the colour Atlas of Hartman and Rhode (1980),Kaminski, (2009), Description of medical fungi atlas (Sarah *et al*., 2016).

III. Results

Total of four (4) bread samples sold within anyigba metropolis were subjected to mycological analysis. The physicochemical properties of the bread were determined and recorded. The proximate analysis of the bread was carried out and the results were recorded. The macroscopic and microscopic properties of the fungal isolates were observed and recorded.

Physicochemical properties of the bread samples

Table 1 shows the physicochemical properties of the bread samples this includes the moisture content (%) and pH (%). Ostrich bread had the highest moisture (%) and Yale bread had the lowest. For pH (%), Yale bread had the highest and Ostrich bread had the lowest.

Table 1: Physicochemical properties of the bread samples

Samples	Moisture (%)	pH (%)
Yale bread	33.2	3.98
Centenary bread	33.5	3.94
Ostrich bread	33.7	3.90
Anyigba bread	33.3	3.95

Proximate properties of bread samples

Table 2shows the proximate properties of the bread. This was carried out on the bread before spoilage occurred. The proximate properties include ash content (%), crude fat content (%), protein content (%) and carbohydrate content (%) of the samples.

Table 2: Proximate properties of bread samples

Samples	Ash (%)	Crude fat (%)	Protein (%)	Carbohydrate (%)
A	1.53	6.50	6.30	48.49
B	1.68	6.34	7.11	47.43
C	1.21	5.97	6.74	48.42
D	1.19	5.72	7.50	49.10

Cultural and morphological characteristics of the molds isolated.

Table 3 shows the cultural and morphological characteristics of the molds isolated. Pigment was observed in all the molds. The colony texture or the organisms were characterized with granular, flat, radial grooves, velvety flaky suede-like periphery was observed to have fine radiating extensions.

Table 3: Cultural and morphological characteristics of the molds isolated.

Growth rate	Colonial morphology	Pigmentation	Periphery	Probable organism
Visible growth within 72hrs	Granular,flat radial grooves velvet to flaky surface	Yellow initially then it turns yellow-green. The reverse is colourless to yellow	Entire	*A. Flavus*
Visible growth occurs within 48-72hrs	Suede-like surface	Blue-green. The reverse is colourless to yellow	Entire	*A. Fumigatus*
Visible growth within 48-72hrs	Suede-like to velvety surface	White to yellow and then becomes black suddenly as it ages. The reverse is colourless to yellow	Fine radiating extensions	*A. Niger*
Fast growing organism within 48-72hrs	Velvety to flaky suface	Dark-greenish. The reverse is light orange	Radiating extensions	*Penicillium sp.*

Microscopic characteristics of the isolated organisms

Table 4 shows the microscopic characteristics of the isolated organisms. Hyphae were observed on all the organisms. The conidiophore ranged from smooth walled to rough walled. The conidia ranged from small to large.

Table 4: Microscopic characteristics of the isolated organisms

Hyphae	Conidia	Conidiophores	Probable organism
Ramified, septate and branched	Conidia heads are radiate. Later splitting to form lose 300-400mm columns via biserate with phialide borne directly on the vessicles and globose(3-6um) and echinulate	Conidiophore stripes are hyaline,coarsely roughened and noticeable near the vesicles	*A. Flavus*
Ramified, septate and branched	Conidial heads are typically columnar(often shorter and smaller). Phialides are on the upper two-thirds of the vessicles,uniserate and finely roughened and greenish	Conidiophore stripes are short,smooth-walled conical shaped vesicles	*A. Fumigatus*
Filamentous,septate hyaline hyphae	Conidial heads are large (3mm),radiate and tends to split into several columns with age,biserate and phialides,often septate metulae,conidia and globose to sub-globose and rough-walled	Conidiophore stripes are smooth-walled,hyaline,turning dark towards the vesicles	*A. Niger*
Branched,septate hyaline hyphae	Conidial consists of chains of single cells produced in a basipetal succession from a phialide	Conidiophore are hyaline, smooth or rough walled	*Penicillium sp.*

Descriptions of medical fungi atlas(Sarah, *et al* . ,2016)

IV. Discussion

The percentage moisture content of the commercial bread samples ranged from 33.2 to 33.7 while the pH values were between 3.90 and 3.98. Ostrich bread had the highest percentage moisture content and pH values of 33.7 and 3.90 respectively while Yale bread had the lowest percentage moisture content and pH values of 33.2 and 3.98 respectively. This work is in agreement with Davidson and Branen, (2004) who reported that these fungi isolated from the bread samples have been reported to be capable of producing amylolytic and propionate enzymes as a result of moisture and pH value which could be the major contributor to their spoilage of bread. Kyzlink, (2001) reported that the low pH of the samples may be due to the use of acidic chemical preservatives such as propionates, palmitates or sorbates in bread. High moisture content and low pH make bread susceptible to fungal spoilage. The susceptibility of bread to spoilage by fungi depends on the chemical compositions, pH and moisture content.

The percentage Ash content of the bread samples ranged from 1.19 to 1.68. Centenary bread sample had the highest Ash content while Anyigba bread had the lowest Ash content.

There was a percentage range of 5.72 to 6.50 for the crude fat composition of the bread samples. Yale bread sample had the highest fat content and Anyigba bread sample had the lowest fat content.

The percentage protein in the bread samples ranged from 6.30 to 7.11. Centenary bread sample had the highest protein content and Yale bread has the least protein content.

The carbohydrates content of the bread samples ranged from 47.43 to 49.10. Anyigba bread sample had the highest carbohydrate content and Centenary bread sample had the least carbohydrate content.

The results of the study corroborated very well with the study of Oluwamukomi et al., (2011) in all the parameters (crude protein, crude fat, ash and the carbohydrate content) assessed showing that the these factors could increase the possibility of the presence of these organisms in the bread samples.

Mould growth is the major microbiological factor affecting the shelf life of bread. The presence of these spoilage fungi in significant numbers in commercial bread is a public health hazard as some of them have been known to produce mycotoxins which are injurious to health. Adequate hygiene should therefore be maintained in all the bread making processes to retard the growth of these fungi.

The frequency of occurrence of the fungi in the commercial bread samples showed that *Aspergillus niger* occurred in all the samples studied while *Penicillium sp.* had the lowest frequency of occurrence having been isolated from Ostrich and Centenary bread samples only which is in agreement with Abellana *et al*. (1999) who reported that *Aspergillius sp.* occurred most frequently in the bread samples they studied which contradicts the work of Anon, (2000) who reported that Penicillium species are by far the most common moulds that spoil bread.

Baked products are prone to mould spoilage. The knowledge of the filamentous fungal spoilage of bread will educate the consumers on the public health implications of the growth of the organisms and their toxins in bread and proffer the possible solutions to such spoilage.

References

[1]. Abellana, M. ,Magri, X. ,Sanchis, V. and Ramos, A.J. (1999). Water activity and temperature effects on growth of Eurotium amstelodami, E. chevalier and E. herbaviorum on a sponge cake analogue. International Journal of Microbiology, 52: 97-103.

[2]. Anon, (2000). House hold consumption and expenditure on cereal-based foods. Home Grown Cereals Authority Weekly Diges.18: 2-3.

[3]. Baur, J. (2001). La Boulangerie en Europe. Industries des Cereals. 73: 39-48.

[4]. Banwart, G. J. (2004). Basic Food Microbiology. A VI Publ. Inc., Westport. pp. 505-544.

[5]. Cheesbrough, N. (2008). Mould growth on cake. Biscuit Maker and Plant Baker, 14: 961- 964.

[6]. Davidson and Branen, A.L. (2004). Marcel Dekker, Inc., New York pp. 117-119.

[7]. Jay B.B. and De Boser D. (1996) HACCP models for quality control, journal food protect 40:632-638

[8]. Kaminski, (2009). Colour atlas of fungal description.

[9]. Kent, N.L. (1983). Technology of cereals. Third Edition. Pergamon Press, Oxford.

[10]. Kyzlink, V. (2001). Principles of food preservation. Elsevier Publ., Amsterdam. pp. 247- 370.

[11]. Marin, S. Guynot, Sanchis, M.E. , Arbones, V. and Ramos, A. J. (2002). Aspergillus flavus, Aspergillus niger and Penicillium coryophilumspoilage prevention of bakery product by means of weak-acid preservatives. J Food Sci, 64: 2271

[12]. Oluwamukomi, M.O. , Oluwalana, I.B. and Akinbowale, O. F. (2011). Physicochemical and sensory properties of wheat cassava composite biscuit enriched with soy flour. African Journal Food Science Vol. 5

[13]. Potter, H. and Hotchkiss, I. (2006). Food science 5th edition, CBS publishers and distributors, New Delhi

[14]. Sarah, F. (2016) Descriptions of medical fungi atlas

[15]. Saranraj, P and Geetha, M. (2012) Microbial spoilage of Bakery products and its control by preservatives. Int J Pharm Biol Arch. 3 (1): 38-48.

[16]. Vytrasova, J. , Pribanova, P. and Marvanova, L. (2002). Occurrence of Xerophilic fungi in bakery production. Int. J. Food Microbiology, 72: 91-96.

Azeez Zainab, et. al. "Isolation and Identification of Fungi Associated With the Spoilage of Some Selected Bread Sold Within Anyigba Metropolis." *IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)*, 21(07), 2022, pp. 40-43.