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Microbial Quality and Aflatoxin Levels of Bread and Flour Products Vended in Akure Metropolis, Ondo State, Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: This study evaluated the microbial quality characteristics of bread and flour-made products vended for human consummation in Akure metropolis.
Methods: The sample products including bread, buns, puff puff, meat pie and cake collected from different locations were analysed using standard microbiological methods to enumerate the bacterial and fungal consortia. Macro and micro-morphological identification of the implicated fungi in the food samples were done via standard techniques. The presence and quantity of some aflatoxin types were also investigated using standard techniques.
Results: The fungal organisms enumerated include species of *Fusarium, Aspergillus, Cladosporium*, *Mucor*, *Sacharomyces cerevisiae, Rhizopus* and *Penicillium.* Bacteria consortium implicated in sample products include; *Staphylococcus aureus, Bacillus* sp., *Escherichia coli, Clostridium* sp., *Pseudomonas aeruginosa* and the likes. The levels of aflatoxin B1 and B2 produced were predominantly associated with *Aspergillus flavus* enumerated from bread products which serve as a rider to the aflatoxin contamination in vended flour products.
Conclusion: The toxicity and potency of aflatoxins make them a primary health hazard and as well accountable for losses associated with contamination of processed foods and ready-to-eat foods. It is recommended that bakers should implement the use of heat-treated flour in the production process of ready-to-eat products for human safety.

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1. INTRODUCTION

Most of the foods are products made from flour and the flour used for such is from a raw material known as "Wheat" [1]. Such food includes bread of different kinds which is a staple food all over the world, also including snacks like pie, puff puff, doughnut, sausage, eggroll, peanut, cake and the likes are designated as ready-to-eat (RTE) and in turn for immediate consumption at the point of sale. As much as these foods are consumed, the processes of making them into finished products are manual and mostly by using of hands which could make the flour-made food products liable to unprecedented microbial contamination [2]. Food safety is an integral part of environmental public health. However, there is a very high tendency for the general populace to patronize ready to eat snacks or take away foods [3] and are mainly street-vended foods [4].

Mycotoxins occurring in food commodities are secondary metabolites of filamentous fungi [5]. They added that these fungi affect cereals notably peanuts, corn, wheat, and rice in which wheat is mostly used for baking and confectionaries. Mycotoxins contaminate many types of food crops throughout the food chain. They are produced by the three main genera *Aspergillus*, *Fusarium,* and *Penicillium* during crop growth, harvesting or storage.

Although hundreds of fungal toxins are known, a limited number of toxins have important roles in food safety. Among these toxins, aflatoxins are highly toxic secondary metabolites predominantly produced by the filamentous fungi *Aspergillus flavus* and *A. parasiticus*, in addition to *A. nomius* and *A. tamari*, *A. pseudotamarii* and *A. bombycis*. Among the major known types of aflatoxins are aflatoxin B1, aflatoxin B2, aflatoxin G1, and aflatoxin G2 [6]. The toxins are potent carcinogen, teratogen and mutagens to humans and animals [7], causing damage such as toxic liver damage/inflammation, haemorrhage, oedema, immune system suppression, and hepatic carcinoma [8]. Ghiasian *et al*. [9] have investigated the natural occurrence of aflatoxins in a range of human foods including wheat, rice and barley, and maize. The main microbial challenges associated with bread are fungal infections and subsequent contamination with their derivatives such as aflatoxins including B1, B2, G1, and G2 (AFB1, AFB2, AFG1, and AFG2). The economic inconveniences arising from fungi and microbial toxins have recently elicited wide-ranging studies [10]. Even though the temperature employed in preparing bread most likely did not impact reducing aflatoxins, but triggering enzymes used through the fermentation alone or in blend with heat can end up reducing aflatoxins, which similarly indicates elimination of heat-resistant fermentative sensitive AFs, during the baking process [11].

This study is aimed at ascertaining the microbial quality and the presence of aflatoxins in vended bread and flour products to ensure the consuming populace is free of pathogenic and toxin-producing fungi which pose threats and risks to the health status of humans in the Akure metropolis.

2. STUDY PROTOCOLS AND MATERIALS

2.1Sample Collection

Samples of buns, puff puff, pies and cake were collected from 5 different locations in Akure between the hours of 11 AM and 12: 30 PM on the 10th of July 2019. Also, 5 brands of bread was collected in 4 places and fresh bakery samples of each brand were all collected aseptically using a sterile polythene bag. The total samples of 90 bread and flour products were collected. Samples of bread, pie, puff puff, cake and buns were purchased from vendors in Akure metropolis, Ondo State. The materials were kept in a sterile polythene bags to avoid cross contamination and transported to the Microbiology department laboratory for analysis within 1 hour of collection. Samples which were not analysed immediately were stored at 4 ºC.

2.2 Preparation of Coconut Aflatoxin Media (CAM), Nutrient and Potato Dextrose Agar

Coconut was purchased locally and 100 g of shredded coconut was homogenized for 5 min with 200 ml of hot distilled water. In all cases, the homogenate was filtered through four layers of muslin cloth, and the pH of the clear filtrate was adjusted to pH 7 with 2N NaOH. Agar was added (20 g/L), and the mixture was heated to boiling and cooled to about 50 °C. The pH was checked and adjusted to 7 when necessary. The mixture was then autoclaved for 15 min at 121 ºC, cooled to about 40 to 45 °C, and poured while being stirred into sterile petri dishes. Each plate

contained a single, large, central colony, the reverse side of which was periodically observed under long-wave (365 nm) UV light for blue fluorescence. An un-inoculated plate was taken as control. Nutrient and potato dextrose agar were prepared following manufacturers' specification.

2.3 Serial Dilution of Vended Food Samples

One gram (1 g) portion of each food sample was dissolved in 9ml of sterile distilled water in a conical flask. The flask was shaken vigorously to achieve homogeneity. Thereafter, 1ml of the mixture (stock) mixed with 9 ml of sterile water using sterile syringe and 1ml was introduced into the next 9 ml of sterile water from the previous into the test tubes under aseptic provisions. The process was repeated until the 10^{-5} diluent as described by Chessbrough, [12], as standard pour plate technique was employed and petri plates were incubated for duration of 24 hrs at a temperature of 37 ^{0} C after rocking the plates clock wisely and anti-clock wisely, before solidification of the media. Plates were incubated in inverted position. *Bacterial* and fungal colonies are counted using a colony counter (J-2 PEC MEDICAL, USA) and result duly recorded in colony forming units per millilitre (Cfu/ml) or spore forming unit per millilitre (Sfu/ml) as described by Khusro *et al.* [13]. The number of bacterial colonies was calculated using the formula:

Number of the bacterial colonies of the food samples = (Total bacterial count × dilution factor / Volume of food sample (g))

2.4 Isolation of Microorganisms from Samples

One millilitre (1 ml) of the diluted sample was taken aseptically with the use of a sterile syringe from the 10^{-3} diluent and inoculated aseptically on the agar plates. The plates were then incubated at 37 ^{o}C for 24 h and 28 °C for 72 h for NA and PDA respectively. The plates were observed for growth after appropriate incubation period and the growth were counted and recorded.

2.4.1 Isolation of bacteria

This was done by weighing one gram of each sample and making serial dilution with sterile water under aseptic condition and then pours plating the various dilution factors on nutrient agar (NA) which is prepared by following the manufacturer's specifications. The plates are allowed to cool and incubated at 37 °C for 24 h. Colonies were counted, characterization and further test to identify the bacteria were done.

2.4.2 Isolation of fungi

Fungi are isolated from the samples by plating the dilution factors from the serial dilution on a selective and differential media such as Potato dextrose agar (PDA) as described by Chessbrough, [12].

2.5 Characterisation of Fungal Isolates

Macroscopic, microscopic and biochemical characterisation were used to identify and characterize the fungi isolates.

2.5.1 Morphological and ccultural characterization (microscopic) of fungi isolates

Morphological and cultural characterization of fungal isolates from food samples was performed as described by Karabay *et al*. [14].

2.5.2 Macroscopic characterization of fungi isolates

Colony characterization is a method used to describe the characteristics of an individual colony of fungi growth in a Petri dish which can be used to identify them. Different fungi produce different colonies, colours, shapes, sizes, forms, surface and elevation etc. Yeast colonies are similar in nature to bacterial colony. Plates are critically observed and proper inferences recorded.

2.6 Biochemical Test for Yeasts

2.6.1 Sugar fermentation test

The carbohydrate fermentation test for the identification and characterization of yeast from food samples were performed as described by Chessbrough, [12].

2.6.2 Urease test

This test was done to detect the presence of enzymes that can hydrolyze urea to ammonia and carbon dioxide. Test tubes containing urease agar prepared according to manufacturer's

specification (2.45 g in 100 ml of sterile water) by introducing 10 ml of urease agar into the test tubes using a sterile syringe and was slant to solidify after autoclaving at 121 °C for 15 mins. The organisms were stabbed and streaked on the agar aseptically and urease positive organisms had a colour change from light orange to magenta (pink) within 24 h incubation at 35°C-37 °C.

2.6.3 Germ tube test

Germ tube test is a diagnostic test in which a sample of fungal spores are suspended in animal serum and examined by microscopy for the detection of any germ tubes. It is indicated in colonies of white or cream colour on fungal culture where a positive result indicates presence of *Candida albicans.*

2.7 Aflatoxin Extraction from Food Samples

Aflatoxin extraction was carried out as described by Hontanaya *et al.* [15]. The common clean-up technique used is immuno-affinity column (IAC) chromatography. During sample cleanup, the crude sample extract is applied to the immune-affinity column containing specific antibodies to aflatoxin immobilized on a solid support such as agarose or silica. As the crude sample moves down the column, the aflatoxin binds to the antibody and is retained onto the column. Another washing step is normally required to remove impurities and unbound proteins. This is achieved by using appropriate buffers and ionic strengths. Thereafter, the aflatoxin is recovered by using such solvents as acetonitrile which breaks the bond between the antibody and the aflatoxin.

2.7.1 Quantification of aflatoxins

2.7.1.1 Preparation of stock culture of fungi isolates

Stock culture of the fungi isolates was maintained on PDA tubes and plates. Inoculations were done by mass conidial transfer to the centre of plates, which were then incubated upside down at 28 °C. Each plate contained a single, large, central colony, the reverse side of which was periodically observed under long-wave (365 nm) UV light for blue fluorescence. An un-inoculated plate was observed for reference.

2.7.1.2 Qualitative analysis of aflatoxin

After the appropriate incubation times, the mycelium of each fungal colony was scraped off the Petri dish plate, cut into strips , and homogenized with 5ml of Chloroform. The extracts were applied to TLC plates of silica gel 60G using capillary tubes and were eluted with toluene, chloroform, ethyl acetate, and formic acid (35: 25:25:10). After developing the chromatogram, the plates were viewed under long wavelength ultraviolet light (365 nm) to detect the characteristic fluorescence of Aflatoxin B1 as described by Eldridge *et al.* [16] with slight modifications.

2.7.1.3 Quantification of aflatoxin through chromatographic methods

Quantification of aflatoxins in food samples through chromatographic methods of thin-layer as described by Fallah *et al.* [17]; Maslowska *et al.* [18] and high performance liquid chromatography was performed as described by Quiles *et al.* [19]. The HPLC technique was used for aflatoxin quantification in food samples according to the method described by Papp *et al.* [20].

2.8 Analysis of Data

The numerical data obtained from the investigation were subjected to analysis of Variance (ANOVA) and duncan's new multiple range test using statistical packages for social sciences (SPSS) 20.0 version. Differences were considered significant at $p \leq 0.05$.

3. RESULTS

From the flour-made product samples, 54 bacterial isolates and 33 fungal isolates were isolated, as well as 65 bacteria and 48 fungi were also isolated from the bread product samples. Among the vended flour product samples, pie (D1) had the highest bacterial count of 9×10^3 CFU/ml while another pie sample had the least bacterial count of 1×10^3 CFU/ml. Cake (C3) had the highest fungal count of 9×10^3 SFU/ml while 3 samples of pie coupled with a sample of cake (C2) all had the least fungal count of 1×10^3 SFU/ml as shown in Table 1.

3.1 Total Microbial Count of Vended Flour-Made Products

Total bacterial count ranged from 0.7×10^5-5.5×10^5 CFU/100ml while total fungal count ranged

from 0.1 × 10^5-6.2×10^5 SFU/ml of the microbial counts from the bread product samples collected from vendors as displayed in Table 2.

3.2 Total Microbial Count of Bread Samples from Local Bakeries

Of the bread samples collected from bakeries, brand D had the highest bacterial count of 6×10^4 CFU/ml and B and E had the least at 1×10^4 CFU/ml. No fungal growth was observed in the bread samples collected from bakeries in Akure metropolis as detailed in Table 3.

3.2.1 Biochemical profile of bacterial organisms from the vended flour-made products

Staphylococcus aureus and *Staphylococcus epidemidis* both had the highest percentage occurrence of 25.71 % as *Proteus* sp, *Pseudomonas aeruginosa* and *Escherichia coli* had the least at 2.86 % occurrence as demonstrated in Table 4. Probable bacterial organisms implicated or enumerated from the collected bread and flour-made product samples are *Bacillus* sp, *S. aureus, S. epidemidis*. *P. aeruginosa*, *Proteus* sp, *E. coli, Clostridium* sp and *Klebsiella pneumoniae* as showed in Table 4.

3.2.2 Morphological profile of fungi from the vended flour product samples

Micro and macro-morphological characteristics of fungi isolated from the vended flour-made product samples includes; *Microsporium versicolor, Candida albicans, Geotrichum candidum, Mucor irregularis, Penicillium* sp, *Rhizopus stolonifer, Aspergillus flavus, Fusarium proliferatum, Candida glabrata* and *Saccharomyces cerevisiae* as detailed in Table 5.

3.2.3 Sugar fermentation profile of yeast from vended flour-made products

Sugar/carbohydrate fermentation outcome of yeast isolates from the flour-made products and bread brand samples for verification of suspected yeast isolates revealed 8 of the yeast isolates (66.7%) were confirmed as *S. cerevisiae* while 3 (25 %) were confirmed as *C. albicans* and 1 (8.3 %) was confirmed as *C. glabrata* as juxtaposed in Table 6.

Table 1. Microbial count (10^{-5}) of vended bread product samples

Bread product	Total bacterial count (Cfu/ml)	Total fungal count (Sfu/ml)
A1	0.9 × 10^5	3.0 × 10^5
A2	1.2 × 10^5	0.2 × 10^5
A3	2.1 × 10^5	0.7 × 10^5
A4	1.1 × 10^5	0.4 × 10^5
B1	3.8 × 10^5	6.2 × 10^5
B2	4.0 × 10^5	5.6 × 10^5
B3	3.4 × 10^5	4.2 × 10^5
B4	4.7 × 10^5	5.0 × 10^5
C1	3.1 × 10^5	0.2 × 10^5
C2	3.2 × 10^5	0.5 × 10^5
C3	2.5 × 10^5	0.1 × 10^5
C4	2.1 × 10^5	0.3 × 10^5
D1	5.1 × 10^5	0.8 × 10^5
D2	4.0 × 10^5	3.3 × 10^5
D3	4.1 × 10^5	0.6 × 10^5
D4	5.5 × 10^5	1.1 × 10^5
E1	0.7 × 10^5	0.1 × 10^5
E2	Nil	2.0 × 10^5
E3	3.0 × 10^5	0.2 × 10^5
E4	2.1 × 10^5	0.1 × 10^5

Key: CFU= Colony forming per unit SFU= Spore forming per unit A= Buns B= Puff puff C= Cake D= Pie E=Meat pie Nil = No growth. Names of breads are in codes to protect to protect the brand name

Table 2. Microbial load of vended flour-made products

Sample	Total aerobic bacterial count (Cfu/ml)	Total fungal count (Sfu/ml)
A1	4.5×10^4	3×10^3
A2	3.2×10^4	5×10^3
A3	4.7×10^4	5×10^3
A4	2.8×10^4	2×10^3
B1	1.5×10^4	3×10^3
B2	2.7×10^4	2×10^3
B3	3.5×10^4	2×10^3
B4	7.3×10^4	2×10^3
C1	6×10^3	2×10^3
C2	4×10^3	1×10^3
C3	3.0×10^4	9×10^3
C4	1.7×10^4	5×10^3
D1	9×10^3	4×10^3
D2	4×10^3	1×10^3
D3	6×10^3	1×10^3
D4	1×10^3	1×10^3

Key: CFU= Colony forming per unit SFU= Spore forming per unit A= Buns B= Puff puff C= Cake D= Pie E=Meat pie. Names of breads are in codes to protect to protect the brand name

Table 3. Microbial count of bread collected from local bakeries

Bread Product	Total bacterial count (Cfu/ml)	Total fungal count (Sfu/ml)
A	2×10^4	Nil
B	1×10^4	Nil
C	3×10^4	Nil
D	6×10^4	Nil
E	1×10^4	Nil

Key: CFU= Colony forming per unit SFU= Spore forming per unit A= Buns B= Puff puff C= Cake D= Pie E=Meat pie. Names of breads are in codes to protect to protect the brand name. Nil = No growth

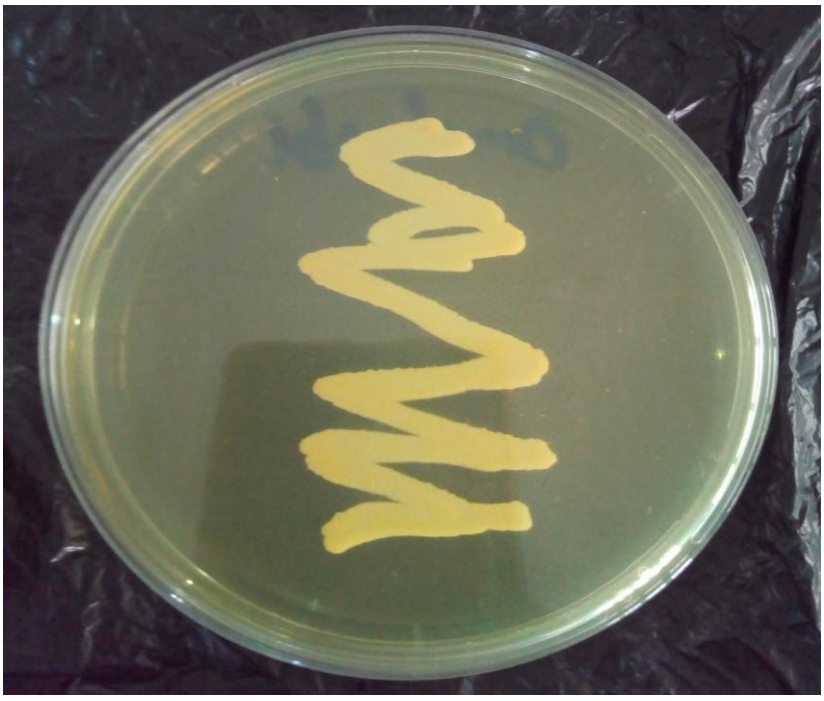

Plate 1. Macroscopic view of *Microsporum versicolor* from vended flour-made products

Table 4. Biochemical characterization of bacterial isolates

S/N	Glu	Su	La	Fr	Ma	Ga	Man	Gas	Coa	Gram stain	Percentage Occurrence (%)	Suspected organisms
1	+	+	+	+	+	+	+	+	+	-ve cocci	25.71	*Staphylococcus aureus*
2	+	+	+	+	+	+	–	+	–	-ve cocci	25.71	*Staphylococcus epidermidis*
3	+	+	+	+	+	+	+	+	+	+ve rods	17.14	*Bacillus cereus*
4	–	–	–	+	–	+	+	+	–	-ve rods	11.43	*Pseudomonas aeruginosa*
5	+	+	+	+	+	+	+	+	-	-ve rods	8.75	*Klebsiella pneumoniae*
6	+	–	–	–	–	+	–	+	–	-ve rods	2.86	*Proteus mirabilis*
7	+	+	+	–	–	+	+	+	–	-ve rods	2.86	*Escherichia coli*
8	+	–	–	+	+	–	+	–	–	+ve rods	2.86	*Clostridium* species

Keys: Gl= glucose, Su= sucrose, La= lactose, Fr= fructose, Ma= maltose, Ga=Galactose, Mn= mannitol, Coa=Coagulase, Cat=Catalase

Table 5. Micro and macro-morphological characteristics of fungi isolated from the vended flour product samples

S/N	Macroscopic Characteristics	Microscopic Characteristics	Reverse Side Colours	Suspected Organism
1	Colonies are flat with a powdery texture	Macroconidia are symmetrical in shape with rounded ends.	Yellow	*Microsporium persicolor*
2	Whitish cream colonies with no true mycelium	Colonies are loose budding cells with round edges	White	*Candida albicans*
3	Colonies flat, white to cream, dry and fine pigment	Hyaline arthroconidia with fragmented hyphae	Pale yellow	*Geotrichum candidum*
4	Fluffy cotton candy appearance	Broad hyphae and non septate	White	*Mucor irregularis*
5	Freely branched greenish mycelium	Long erect conidiophores with round-shaped conidia	Pale yellow	*Penicillium* sp
6	White mycelia growth	Non-Septate with brown sporangiophore	White	*Rhizopus stolonifera*
7	Fastidious grayish white mycelium with yellowish-green centers	Long coarse conidiophores with radiate conidial heads	Light yellow	*Aspergillus flavus*
8	Yellowish pink creamy colonies	Cylindrical to ovoid conidia	White	*Fusarium proliferatum*
9	Whitish cream colonies with no true mycelium	Loose budding cells colonies with round edges	White	*Candida albicans*
10	Flat, smooth, moist, glistening, cream colour	Unicellular and globose conidiophores	Creamy white	*Saccharomyces cerevisiae*

Table 6. Sugar/carbohydrate fermentation characterization of yeast isolates

Sample	Glu	Su	La	Ma	Ur	GT	Suspected organism
A	+	–	–	+	+	–	*Saccharomyces cerevisiae*
B	–	+	+	+	–	–	*Saccharomyces cerevisiae*
C	+	+	+	–	+	–	*Saccharomyces cerevisiae*
D	+	+	+	+	+	–	*Saccharomyces cerevisiae*
BA	+	+	+	–	+	–	*Saccharomyces cerevisiae*
D	+	+	+	+	+	+	*Candida albicans*
BB	+	+	+	–	+	–	*Saccharomyces cerevisiae*
BC	+	+	+	+	+	+	*Candida albicans*
BD	+	+	+	–	+	–	*Saccharomyces cerevisiae*
C	+	+	+	+	+	+	*Candida albicans*
BE	+	+	+	–	+	–	*Saccharomyces cerevisiae*
A	+	–	–	–	–	–	*Candida glabrata*

Keys: Glu= glucose Su= sucrose La= lactose Ma= maltose Ur= urease GT= germ tube test A= buns; B= puff puff; C= cake; D= Pie; BA= Bread 1; BB= Br ead 2; BC= Bread 3; BD= Bread 4; BE= Bread 5

3.2.4 Qualitative aflatoxin production profile of vended flour-made products

Qualitative analysis of aflatoxin B1 in flour-made and bread product samples (vended and freshly collected from bakeries) showed that only vended bread product samples had aflatoxin B1 production on coconut aflatoxin media (CAM), further confirmed on thin layer chromatography (TLC) plate while aflatoxin B1 production was suspected or confirmed in all flour-made products as demonstrated in Table 7.

3.2.5 Aflatoxin B levels of bread samples

Quantitative aflatoxin levels in suspected bread product samples demonstrated concurrent levels of 5.487395 µg/ml (aflatoxin B1) and 5.605042 µg/ml (aflatoxin B2) as displayed in Table 8.

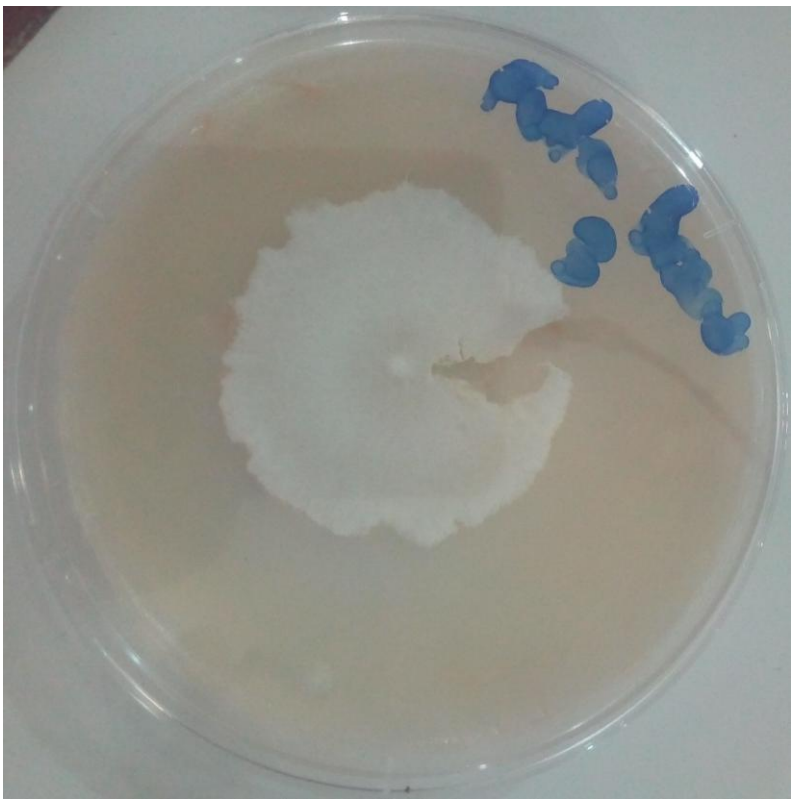

Plate 2. Macroscopic view of *Saccharomyces cerevisiae* from vended flour-made products

Table 7. Qualitative aflatoxin tested in samples

Samples/Isolates	Aflatoxin B1 production	Aflatoxin B1 production(confirmatory Test)
	CAM Blue Fluorescence Visible at 365nm	TLC Plate Blue Fluorescence Visible at 365nm
1	Absent	–
2	Absent	–
3	Absent	–
4	Suspected	+
5	Absent	–

Keys: CAM: Coconut aflatoxin Media; TLC: Thin layer chromatography 1=Pie; 2=Cake; 3=Puff puff; 4=Bread; 5=Buns

Table 8. Quantitative aflatoxin levels in suspected samples

Sample	Aflatoxin	Levels (µg/mg)
Bread	B1	5.487395
Bread	B2	5.605042

Keys. B1= Aflatoxin B1 ;B2= Aflatoxin B2

4. DISCUSSION

The evaluation of the microbial quality and safety of bread and flour-made products vended for human consumption in Akure metropolis was detailed in this study. The high microbial load recorded in flour-made product samples and the bread samples can be attributed to the level of unfavourable environmental exposure of these products as well as the conduct of routine of the people living in those areas; insanitary custom of the handlers as well as the unprocessed resources used in the processing of these products which can also increase the upshot of the implicated microbial loads in the flour-made and bread sample products in alliance with Barro *et al.* [21]. The high bacterial count yielded by the meat pie samples maybe due to unsavoury hygienic conditions in terms of usage of apron, handling of the meat pie and covering of hair, not appropriately followed by any of the hawkers noticed in the evaluated metropolis. The presence of these organisms can be linked to a number of factors; such as improper handling and processing, use of contaminated water during washing, cross contamination from other raw materials or the use of dirty processing utensils like knives and trays as supported by Clarence *et al.* [22]. The high fungal contamination observed in the vended cakes may be owed to the poor post-baking handling and storage conditions of the vendors which pose greater risk of various microbial contaminations like bacteria, mold and spores in them as augmented by Al-fuad *et al.* [23]. Another rationale for high fungal spoilage of flour-made products is that since the recommended limit for yeast and mold in flour-made products is 10^5 CFU/g according to world food program, (WFP) [24]. Molds can produce mycotoxin and higher absorption of these fungal organisms can cause deterioration of food and food-borne illness as maintained by Bullarman, [25]. Yeast and mold counts were found to fall relatively below the recommended limit in all food samples except puff puff samples which showed higher bacterial yield than the 10^5 CFU/g recommended limit of fungal count.

Relatively high bacterial and fungal count of locally-vended bread products can be attributed to relatively high moisture content which encourages the growth and development of mold and bacteria on the bread as any bread kept open or partially sealed can be infected and deteriorated by airborne fungus, molds and bacteria as maintained by Al-fuad *et al.* [23]. Decreased microbial count yielded by branded bread samples from branded bakery producers was because the bread processing processes are well monitored and regulated by various food safety authorities as supported by Al-fuad *et al.* [23].

From the results of the bacteriological analysis of the vended flour-made sample products carried out, The presence of *Staphylococcus aureus*, *Salmonella* sp, *Klebsiella* sp and many *Bacillus* sp and *Escherichia coli* suggested the possibility of faecal contamination of the food products like puff puff samples collected in this investigation due to poor sanitation practices of road sellers. On the other hand, these organisms are ecological contaminants and likewise

opportunistic pathogens that have been implicated to cause food-borne infections which may result to vast cost-effective losses. The presence of *Bacillus cereus* is due to the fact that it is a copious spore-former in soil, air and water, consequently can be present in the food samples as a result of exposure of the product on the road side. This inference is in agreement with Oluwafemi and Simisaye, [26]; Clarence *et al.* [22]; Okonko *et al.* [27] as they isolated similar organisms from sausages, meat pie and sea foods, respectively.

Bacteria consortia like *Bacillus* species, *Escherichia coli*, *Salmonella* species, and *Staphylococcus aureus* may also taint and cause ropiness of the bakery food items while the products are manually sliced, wrapped and packaged and distributed as stated by Al-Defiery and Merjan, [28]. A proper vending practice like cleanliness status of retailer or storekeeper was found to link to the microbial status of different food products that are further sturdily held to be connected with the educational standing of the vendors as supported by Abdulkareem *et al.* [29].

The presence of *Staphylococcus aureus* is largely as a result of human contact being a typical inhabitant of the hands and this suggest deprived sanitized practices of the vendors [30]. The isolation of *Klebsiella* spp, *staphylococcus aureus, E. coli*, and *Salmonella* spp, corroborate the findings of Oranusi *et al*. [31]; Taulo *et al*. [32] in which these organisms were implicated in ready-to-eat foods. Biochemical results observed in this study also aligns with Olutiola *et al.* [33]. Bibeki, [34] also maintained that the contamination of food items by specific species of microorganisms is largely due to the presence of these organisms and their entrance into food or beverage as a result of poor hygiene and sanitation. The principal occurrence of *Saccharomyces cerevisiae* implicated in both flour-made vended products and bread sample products is due to it being non-pathogenic as well as being the major ingredient in the processing processes of the investigated ready-to-eat food samples such as flour and sugar.

Findings of the fungal consortium of vended flour-made products and bread sample products correlates with Hassan *et al.* [35] who isolated mostly *S. cerevisiae*, *Candida* species, and the likes from puffpuff and other ready-to-eat products which explains their culpability as public health hazard and food-borne illness for unsuspecting and ignorant consuming population. Detection of aflatoxins B1 and B2 in bread sample products is analogous to the observation of Quiles *et al.* [19] who detected aflatoxins and *Aspergillus flavus* reduction in loaves of bread through the use of natural ingredients. These results reveal that several control procedures should be used to reduce microbial load and mycotoxins in flour and flour-based products. These include the prevention of microbial establishment and growth within the flour mill and throughout the production chain through proper sanitation of production equipment, stringent temperature control during baking, testing of incoming wheat and the resulting flour, and hygienic packaging which are meant to improve the shelf life

5. CONCLUSION

This study has been able to discover the microbial quality of various bread produced by different manufacturers and flour-made vended ready-to-eat snacks in the cosmopolitan city of Akure, Nigeria. The quality of the bread sold by vendors of different locations established the fact that many types of microorganisms are introduced into the food products at the intermediary stage between vendors and final consumers in which contains some organisms which are pathogenic such as *Salmonella species, Escherichia coli*, and organisms that are carcinogenic to human health such as *Aspergillus flavus* which produces aflatoxins and when consumed overtime with high aflatoxin load can cause aflatoxicosis. The majority of organisms present in the food are what can be referred to as normal flora (*Saccharomyces cerevisiae*), handling personnel introduced microorganisms, packaging introduced microorganisms, and storage environment introduced microorganisms.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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