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Original Research Article

Assessment and Determination of Fungal Load and Species Isolated from Cake Samples Sold in Kano, Northern Nigeria

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Abstract: The study was aimed to assess and determine fungal load and species isolated from cake samples sold in Kano, Northern Nigeria. A qualitative test was performed to ascertain the types of fungi that were present in the cake samples using standard microbiological techniques. Data obtained was expressed as Mean ±SD and compared with ICMSF Standard. A total of four hundred and fifty (450) cake samples from three selling points (markets, shops and bakeries) of the three senatorial districts of Kano State were collected and analyzed. The mean fungal counts of the cake samples analyzed ranged from 5.35×10^1 - 36.1×10^2cfu/g (Nassarawa- Tofa). Colonial morphology showed the presence of four fungal genera (*Aspergillus, Rhizopus, Mucor and Penicillium*). The frequency of occurrence of fungal species isolated from the cake samples; were as follows *Aspergillus fumigatus* 6 (11.5%), *Aspergillus niger* 29 (55.7%), *Rhizopus* 7 (13.5%), *Mucor* spp 5 (9.6%), and *Penicillium* spp 5 (9.6%). *Aspergillus niger* has the highest frequency of 29 (55.7%), while *Mucor* spp has the lowest frequency of 5 (9.6%). A total 52 (11.55%) fungal species were isolated altogether from the cake samples. All the fungal species isolated in this study were extensively contaminants. It is concluded that some cakes sold in Kano, Northern Nigeria contain some fungi.

Keywords: Cake, Mesophilic fungal, Kano, *Aspergillus* sp.

INTRODUCTION

Cake is any form of sweet desert that is typically baked [1]. In its oldest forms, cakes were modification of bread but cover a wide range of preparations that can be simple or elaborate and share features with other deserts such as pastries, custard and pies. Typical cake ingredients are flour, sugar, eggs, butter or oil, a liquid and leavening agents such as baking soda and or baking powder. Cake is often served as a celebratory dish on ceremonial occasions for example weddings, anniversaries, and birthdays [2]. Fungi include mold and yeasts which can adapt to various conditions than other microorganisms. They have high tolerance for acidic condition and are more often responsible for food spoilage than for food-borne illness [3].

A wide variety of fungi, including species of *Rhizopus, Alternaria, Penicillium, Aspergillus*, and *Botrytis* spoil foods. Since fungi grow readily in acidic as well as low moisture environments, fruits and breads are more likely to be spoiled by fungi than by bacteria. *Aspergillus flavus* infects peanuts and other grains, producing aflatoxin, a potent carcinogen monitored by the food and drug Administration [4]. Factors affecting the growth of germs in food and constant incarceration also determine the nature of the defects and any health risks posed for convenience; which can be divided into groups; Intrinsic factors: Intrinsic factors of a food include nutrients, growth factors, and inhibitors or (antimicrobials), water activity, pH, and oxidative potential. The influence of each factor on nutritional system are inter related and affects microbial growth in combination, for good or bad [5,6]. Extrinsic factors which include environmental factors like temperature, Relative humidity and gases, Implicate factors Include Specific growth, Synergism, Antagonism

and commensalism. Ethical competition among the microorganism cause changes of physical and chemical structure of the food and spoilage of the food [7-9]. Many of the foods sold in our communities are highly contaminated with fungi, revealing that a large fraction of foods sold near polluted environments are prone to contamination by microorganisms. Bakery products are subject to the microbiological spoilage problems affecting foods. If moisture content is kept below 12 to 14 percent (depending on the composition), growth of yeast, bacteria and molds are completely prevented. In a recent assessment by the World Health Organization, it was found that poor food handling practices are among the major causes of food borne disease, in addition to poor sanitation and contaminated water supplies [10]. The study was aimed to assess and determine fungal load and species isolated from cake samples sold in Kano, Northern Nigeria

MATERIALS AND METHOD

Fungal Count of the Cake Samples

Twenty five (25) gram of each cake sample was homogenized in 225ml diluents (sterile peptone water), and was labeled as 10^{-1} dilution. From this, 1 millimeter is transferred into another test tube containing 9ml of the diluents and labeled as 10^{-2} dilution. The same procedure was repeated up to 10^{-5} dilution [11]. One (1) ml from each of dilution was transferred into appropriately labeled Petri dishes. In the dish, Potato Dextrose Agar (PDA) was poured and also allowed to cool and solidify. Plates were then incubated at 37 ^{0} C for 24 hours. Following the 24 hours incubation, plates containing 30-300 colonies were counted and Multiplied by the dilution factor to get the number of colony forming unit (cfu) per gram of the sample [11].

Isolation and Identification of Fungal Species

Colonies were picked from appropriate the PDA plates which were maintained/preserved on a freshly prepared PD slants. This was done by picking a colony from an appropriate plate using a sterile wire loop and streaked on PDA slants. It was incubated for 72 hours at 25^{0}C [11]. A drop of lactophynol (cotton blue) was placed on a clean slide and a sterile needle was used to introduce a small amount of the fungi into the stain. Cover slip was gently applied with little pressure to eliminate air bubbles. The slides were then mounted on a microscope and observed with objective lens ×10 and ×40 for confirmation. The fungal species were identified by observing there colonial and morphological structures [11].

Data Analysis

The mesophilic fungal loads were expressed as Mean ±SD using descriptive statistics and were compared with International Commission on Microbiological Specifications for Foods (ICMSF) [12] standard.

RESULTS

Enumeration of Fungi

From the results, letter A,B,C,D and E represents the different cake samples of the five local governments areas of Kano Central enrolled the study, the highest mean fungal counts was found in Ungogo ($37.8X10^{2}$ ± 15.7) local government while the lowest mean fungal counts was found in Nasarawa ($4.9x10^{1}$ ± 0.6) local government. All the mean fungal counts were within the acceptable limits of ICMSF [12].

Table 1: Showing the Mean Fungal Counts in cfu/g of Cake Samples Sold in Some Local Governments Areas of Kano Central Senatorial District

SN	Madobi	Count	Fagge	Count	Ungogo	Count	Kumbotso	Count	Nassarawa	Count	ICMSF Std per g
1	A1	$7.5X10^{1}$	B1	$1.0X10^{2}$	C1	$1.2X10^{3}$	D1	$4.9X10^{1}$	E1	$9.6X10^{1}$	$0\text{-}10^{3}$
2	A2	$4.3X10^{1}$	B2	$9.1X10^{1}$	C2	$1.2X10^{2}$	D2	$9.6X10^{1}$	E2	$5.6X10^{1}$	
3	A3	$6.5X10^{1}$	B3	$1.0X10^{2}$	C3	$1.3X10^{3}$	D3	$4.2X10^{2}$	E3	$4.7X10^{1}$	
4	A4	$4.7X10^{1}$	B4	$4.7X10^{1}$	C4	$1.3X10^{2}$	D4	$3.3X10^{1}$	E4	$4.0X10^{1}$	
5	A5	$4.5X10^{1}$	B5	$1.4X10^{1}$	C5	$8.1X10^{1}$	D5	$2.7X10^{1}$	E5	$3.5X10^{1}$	
6	A6	$4.3X10^{1}$	B6	$3.8X10^{1}$	C6	$7.5X10^{2}$	D6	$5.4X10^{1}$	E6	$5.0X10^{1}$	
7	A7	$5.5X10^{1}$	B7	$9.4X10^{2}$	C7	$3.8X10^{1}$	D7	$7.8X10^{1}$	E7	$4.9X10^{1}$	
8	A8	$3.0X10^{1}$	B8	$4.7X10^{1}$	C8	$4.1X10^{1}$	D8	$2.7X10^{1}$	E8	$2.7X10^{1}$	
9	A9	$5.0X10^{1}$	B9	$3.6X10^{1}$	C9	$8.0X10^{1}$	D9	$1.1X10^{2}$	E9	$5.4X10^{1}$	
10	A10	$8.0X10^{1}$	B10	$6.1X10^{1}$	C10	$9.0X10^{1}$	D10	$4.5X10^{1}$	E10	$4.1X10^{1}$	
Mean		$5.4X10^{1}$		$14.8x10^{1}$		$37.8X10^{2}$		$9.4x10^{1}$		$4.9x10^{1}$	
±S.D		0.5		8.9		15.7		3.7		0.6	

From the results, letter F,G,H,J and K represents the cake samples of Kano North enrolled in the study, among the five local governments areas of Kano North enrolled in the study, the highest mean fungal counts was found in Tofa ($36.1x10^2 \pm 17.9$) local government, while the lowest mean fungal counts was found in Dawakin Tofa ($18.9x10^1 \pm 12.9$). All fungal counts were also within the acceptable limits of ICMSF [12].

Table 2: Showing the Mean Fungal Counts of Cake Samples in cfu/g of Some Local Governments Areas of Kano North Senatorial District

SN	Dawakin Tofa	Count	Tofa	Count	Bichi	Count	Danbatta	Count	Rimin Gado	Count	ICMSF Std per g
1	F1	$9.5X10^1$	G1	$4.9X10^1$	H1	$1.10X10^2$	J1	$6.3X10^1$	K1	$1.21X10^2$	$0\text{-}10^3$
2	F2	$8.4X10^1$	G2	$5.8X10^1$	H2	$4.5X10^1$	J2	$1.06X10^2$	K2	$8.2X10^1$	
3	F3	$5.5X10^1$	G3	$7.1X10^1$	H3	$6.5X10^1$	J3	$5.8X10^1$	K3	$8.9X10^1$	
4	F4	$9.4X10^1$	G4	$1.0X10^2$	H4	$1.2X10^3$	J4	$7.9X10^1$	K4	$9.0X10^1$	
5	F5	$1.3X10^3$	G5	$1.5X10^3$	H5	$5.7X10^1$	J5	$8.8X10^1$	K5	$4.1X10^1$	
6	F6	$5.6X10^1$	G6	$1.2X10^2$	H6	$7.9X10^1$	J6	$1.3X10^3$	K6	$1.4X10^2$	
7	F7	$4.0X10^1$	G7	$1.0X10^2$	H7	$1.2X10^3$	J7	$5.8X10^1$	K7	$1.3X10^3$	
8	F8	$4.6X10^1$	G8	$1.1X10^2$	H8	$1.2X10^2$	J8	$4.5X10^1$	K8	$5.0X10^1$	
9	F9	$6.4X10^1$	G9	$1.4X10^3$	H9	$9.5X10^1$	J9	$5.6X10^1$	K9	$8.5X10^1$	
10	F10	$5.0X10^1$	G10	$1.4X10^2$	H10	$4.4X10^1$	J10	$1.1X10^3$	K10	$4.5X10^1$	
Mean		$18.9x10^1$		$36.1x10^2$		$29.9x10^2$		$28.6x10^2$		$20.4x10^1$	
±S.D		12.9		17.9		14.9		14.6		12.1	

From the results (Table 3), letter M,N,P,Q and R represents the cake samples of Kano South enrolled the study, among the five local governments of Kano South, the highest mean fungal count was found in Garko ($34.35x\ 10^2 \pm 16.71$) while the lowest mean fungal counts was found at Warawa ($11.10x\ 10^1 \pm 3.2$) local government. All the mean fungal counts were within the acceptable limits of ICMSF [12].

Table 3: Showing the Mean Fungal Counts of Cake Samples in cfu/g of Some Local Governments Areas of Kano South Senatorial District

SN	Gaya	Count	Garko	Count	Dawakin kudu	Count	Warawa	Count	Karaye	Count	ICMSF Std per g
1	M1	$1.0X10^2$	N1	$6.7X10^1$	P1	$1.12X10^2$	Q1	$1.20X10^2$	R1	$6.2X10^1$	$0\text{-}10^3$
2	M2	$1.3X10^3$	N2	$9.0X10^1$	P2	$8.2X10^1$	Q2	$6.0X10^1$	R2	$7.4X10^1$	
3	M3	$1.2X10^2$	N3	$5.5X10^1$	P3	$5.8X10^1$	Q3	$5.4X10^1$	R3	$1.00X10^2$	
4	M4	$7.0X10^1$	N4	$1.41X10^2$	P4	$1.31X10^2$	Q4	$9.3X10^1$	R4	$8.6X10^1$	
5	M5	$5.2X10^1$	N5	$1.00X10^2$	P5	$1.43X10^3$	Q5	$6.4X10^1$	R5	$6.6X10^1$	
6	M6	$1.4X10^2$	N6	$1.30X10^2$	P6	$1.16X10^2$	Q6	$2.5X10^1$	R6	$1.32X10^2$	
7	M7	$6.5X10^1$	N7	$8.5X10^1$	P7	$1.27X10^2$	Q7	$5.0X10^1$	R7	$1.08X10^2$	
8	M8	$1.4X10^3$	N8	$1.35X10^3$	P8	$1.08X10^2$	Q8	$1.29X10^2$	R8	$5.9X10^1$	
9	M9	$4.7X10^1$	N9	$7.7X10^1$	P9	$5.4X10^1$	Q9	$1.35X10^2$	R9	$1.40X10^3$	
10	M10	$5.2X10^1$	N10	$1.34X10^3$	P10	$1.18X10^2$	Q10	$3.8X10^2$	R10	$1.39X10^2$	
Mean		$32.9x10^2$		$34.4x10^2$		$23.4x10^2$		$11.1x10^1$		$22.3x10^2$	
±S.D		16.7		16.7		13.3		3.2		13.1	

Fungal Identification

The fungal identification of the cake samples examined is presented in Table 4. The four fungal genera are as follows; *Aspergillus* spp, *Mucor* spp, *Penicillium* spp and *Rhizopus* spp respectively. The fungi were identified based on their microscopic and colonial morphology.

Table 4: Showing Colony Morphology and Microscopic Examination of Fungal species Isolated

Code	Colonial Morphology	Microscopic Examination	Identified fungi
IS_1	Very common colors of colony (black & white)	Conidiophores terminate in a ball like structure	*Aspergillus* spp
IS_2	Cream white/large fluffy white colonies almost covering the whole surface	Broad hyphae which are scarcely septate having long sporongiophore	*Mucor* spp
IS_3	Large fluffy white colonies almost covering the whole surface	Branched hyphae with phialides grouped in a brush-like cluster	*Penicillium* spp
IS_4	Large fluffy white milky colonies which later turns black as culture ages	Presence of stolon and rhizoid with sporangia above rhizoid	*Rhizopus* spp

Prevalence of Fungal Isolates

The frequency of occurrence of fungal organisms isolated from all the cake samples were presented in Table 5, a total of five fungal species were isolated in the cake samples with prevalence of (11.54%) *Aspergillus fumigatus*, (55.76%) *Aspergillus niger*, *Rhizopus* (13.46%), *Mucor* specie (9.61%), and *Penicillium* (9.61%).

Table 5: Showing the Frequency of Occurrence of Fungal Isolates in the Cake Samples

S/N	Isolates	No. of Fungi Isolated	Frequency (%)
1	*Aspergillus fumigatus*	6	11.5
2	*Aspergillus niger*	29	55.8
3	*Rhizopus* spp	7	13.5
4	*Mucor* spp	5	9.6
5	*Penicillium* spp	5	9.6
	Total	**52**	**100**

DISCUSSION

The findings of this study revealed that the mean fungal load of the three Senatorial districts of Kano State ranged from 5.35 x 10^1 - 36.1 x 10^2cfu/g Nassarawa- Tofa local government area. All the mean fungal counts were within the acceptable limits of ICMSF [12]. The results clearly highlights that more sanitary practices are been adopted in Kano central senatorial districts than Kano north and south senatorial districts. *Aspergillus* spp was the most predominant of the fungal species isolated from the cake samples. Contamination of the cakes by fungi could be as a result of poor handling practices in production process, food supply chain, storage conditions, distribution, marketing practices and transportation. Generally, fungi that cause spoilage could be considered toxigenic or pathogenic [13]. All the fungal species isolated in this study are mostly contaminants. This resembles the result of Easa [14] in which different *Aspergillus* specie were isolated from traditional fast foods. Findings were also in accordance with Onyeze *et al*., [15] who also isolated *Mucor* spp (6%), *Aspergillus* spp (9%), *Rhizopus* spp (15%) and *Penicillium spp* (33%) respectively in Zea mays. Oranusi *et al*., [16] in Imo State, Nigeria, also accessed ready to eat foods, obtained species of fungi including *Aspergillus fumigatus, Aspergillus niger, Penicillium* spp *and Mucor* spp, the mean total fungal count from site 1 was 6.0×10^2 to 7.3×10^4 while Site 2 had fungal plate count of 9.0×10^3 to 9.3×10^6 respectively. Madueke *et al*., [17] determined a mean fungal count ranging from 1.5×10^5 cfu/g (yam) to 6.0×10^5 cfu/g (fish), the organisms encountered include *Mucor, Penicillium* spp, *Aspergillus niger, Aspergillus flavus, Fusarium* sp *and Rhizopus stolonifer*. Oranusi *et al*., [18] analyzed for some specific pathogens and fungi in a university cafeteria, the fungal counts from the three sites are within 1.0×10^2- 4.0×10^2, three fungal isolates were identified as *Aspergillus niger, Penicillium* spp and *Mucor* spp. Mary *et al*., [19] screened for various filamentous fungi species in samples of maize, rice, cocoa and cocoa-based powder beverage, results indicated a range of filamentous fungi genus including *Aspergillus, Penicillium, Fusarium, Cladosporium* and *Rhizopus* with *Aspergillus* and *Penicillium* dominating most of the samples. Findings of this study were also in accordance with the limit specified by the International Commission of Microbiological Specification of Food [12]. Contrary to these findings were those of [20, 21]. Contradictory to this findings was also that of Muhammad *et al*., [22] in mycological studies of fungi ascertained in apparently diseased sweet orange (*Citrus sinensis*) and banana (*Musa sapientum*) sampled from various points in Kara market in Sokoto Metropolis revealing that the most predominant fungi isolated from sweet orange was *Cladosporium* spp (40%), *Fusarium* spp (30%), *Alternaria* spp (20%) and *Chrysonillia* spp (10%) while the most predominant fungi isolated from banana was *Fusarium* spp (50%), *Mucor* spp (30%) and *Rhizopus* spp (20%).

CONCLUSION

Of the 450 cake samples analyzed for mesophilic fungal loads and fungal species, the mean aerobic mesophilic fungal counts of the cake samples ascertained ranged from 5.4×10^1-36.1×10^2cfu/g (Nassarawa-Tofa). A total of five fungal species were also isolated in the cake samples with prevalence of (11.54%) for *Aspergillus fumigatus*, (55.76%)

Aspergillus niger, *Rhizopus* (13.46%), *Mucor* specie (9.61%), and *Penicillium* (9.61%). Due to short shelf life of cakes, it is advisable that it should be stored properly and consumed shortly after production so as to curtail foodborne illness. Food regulatory agencies like NAFDAC should ensure high sanitary practices are employed during food production, packaging and transport to sales point.

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