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MICROBIOLOGICAL QUALITY ASSESSMENT OF LOCALLY MADE *FURA* SOLD IN DUTSIN-MA TOWN , KATSINA STATE

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ABSTRACT
The locally made *Fura* spiced ground millet processed and molded into balls for sale and consumption. *Fura* is usually molded into balls by hand during its production, and the hands of the producers could be a source of contamination. Aim of this study was microbiological quality assessment of locally made *Fura* sold in Dutsin-ma, Katsina state. Twenty-five samples of fura were collected total mold and bacterial counts were carried out using pour plate technique, bacteria and fungi were isolated from fura, antibiogram of the isolated organism were carried out. The occurrence prevalence of bacterial species with the highest rate in this study was for *Staphylococcus* spp. and *Streptococcus* spp. (20.6%), while the least rate was (8.8%) for *Klebsiella* spp. and *Bacillus* spp each. The antibiotic susceptibility pattern for the bacterial isolates revealed that most of the organisms were susceptible to the antibiotics except for *Pseudomonas aeruginosa* which is resistant to Chloramphenicol, Sparfloxacin, Ciprofloxacin, Perfloxacin Ofloxacin and Augmentin antibiotics. Fungal occurrence prevalence rate revealed *Aspergillus fumigatus* has the highest rate (38.5%) while *Aspergillus flavus. Aspergillus tereus, Aspergillus niger* and *Aspergillus nidulans* with the least occurrence prevalence rate of (7.7%) each. The fungal species were further tested against some antifungal agents (Ketaconazole, Fluconazole and Greseofulavin), as *Aspergillus flavus* was the only fungal species resistant to the antifungal agents. Locally prepared Fura contains potential pathogenic bacteria and fungi. The producers of 'Fura; should be educated on the basic concept of hygiene in the production processes of 'Fura'.
Keywords: Fura, Locally processed food contamination, Dutsin-Ma, Food Handling.

INTRODUCTION

Fura is the Fulani word for the spiced ground millet processed and molded into balls for sale and consumption. Millet is an important staple for food and nutritional security in semi-arid regions of the world. It is an important staple in several semi-arid and tropical regions of the world with excellent nutraceutical properties as well as ensuring food security in these areas even during harsh environment (Gupta *et al*., 2018). *Fura* is known for several health benefits and some of the health benefits are attributed to its polyphenol and dietary fibre contents (Jideani *et al.,* 2001). The locally made Fura can still be an inanimate vector of infectious microbes and/or toxins. Fura is usually molded into balls by hand during its production, and the hands of the producers could be a source of contamination. Houseflies are also found in large numbers at the production sites and sales outlet. Millet is a general term used for a wide range of cereals that describe several taxonomically divergent species of grass. Millet is thought to be among the first cultivated crops and has been a staple food ingredient in Central and Eastern Asia, Europe (mainly Russia), China, India, and some parts of Africa since ancient times (Okoye, 1992). It is an important food in many developing countries because of its ability to grow under adverse weather conditions such as limited rainfall. In addition, millet has many nutritious and medicinal properties (Obilana *et al.,* 2002).

The fermentation process in Fura is achieved through spontaneous fermentation using indigenous bacteria and yeast inherent in the cereals. However, reports indicate that lactic acid bacteria genera such as Lactobacillus, *Pediococcus*, *Streptococcus* and *Enterococcus* species as well as yeasts such as *Saccharomyces cerevisiae*, *Pichia anomala* and *Candida* species are associated with cereal fermentation (Nche *et al.,* 1994). During fermentation, lactic acid and other organic acids accumulate resulting to a decrease in the pH due to microbial activities thereby inhibiting the growth and survival of spoilage and pathogenic organisms depending on the type of organism and the temperature of the medium. However other organisms have been isolated from Fura. For instance, isolated *Lactobacillus*, *Pediococcus, Streptococcus, Leuconostoc, Enterococcus, Enterobacter aerogenes, Klebsiella pneumoniae, Proteus vulgaris, Enterobacter sakazakii, Serratia liquefaciens, Escherichia coli and Issatchenkia orientalis,* has been reported. Moreover, poor handling of fura during processing, storage and marketing can predispose it to microbial contamination as they are molded into balls by hand during preparation, and storage may be in an unhygienic containers and environment (Adebesin *et al.,* 2001). Also, improper handling and post-fermentation processing such as pounding in mortar, molding and the point of sale can expose the fura product to microbial contamination.

Majority of traditional cereal-based foods consumed in Africa are processed by spontaneous fermentation and are important as weaning foods for infants and as dietary staples for adults. Detailed knowledge of traditional processing is a prerequisite for investigating ways to improve both the nutritional and microbiological qualities of the corresponding product.

In this study, the traditional processing of millet into Fura, a popular millet-based dumpling consumed throughout West Africa, particularly Nigeria, Dutsin-ma, Katsina state was investigated in a range of production units in northern Nigeria. Bacteriology of the processing was also investigated. Processing steps included soaking or steeping the grains, washing, dehulling, wet milling with the addition of aromatic ingredients, dough fermentation (optional), initial moulding, cooking, pounding into sticky cohesive mass and final moulding. The isolated organisms were identified based on morphological, physiological and biochemical characteristics as *Pseudomonas aeruginosa, Klebsiella* spp, *Escherichia coli, Bacillus* spp, *Staphylococcus* spp*, Staphylococcus aureus and Streptococcus* spp were also isolated and identified. The development of starter culture from the dominating organisms is important for the potential production of standardized fura at a commercial, small industrial scale, and for the improvement of its acceptability, microbiological stability and hygienic safety.

The aim of this study was to determine the microbiological quality and antimicrobial susceptibility profile of pathogenic microorganisms in locally made Fura sold in Dutsin-ma, Katsina state.

MATERIALS AND METHODS

Study Area

This study was conducted in Dutsin-ma, Katsina state, Nigeria. The LGA has an area of 527 square km and a population of 169,671 at the 2006 census. Dutsin-Ma has latitude of 12°27'16.13"N and a longitude of 7°29'51.55"E or 12.45448 and 7.497654 respectively.

Sample Collection

Twenty-five gram (25g) of each sample was weighed and introduced into conical flask containing 225ml of distilled water to make a homogenous concentration to form a stock solution. Ten folds serial dilution up to 10^{-4} was made from the stock solution. Then,1ml volume of 10^{-2} was placed in Petri dishes of nutrient agar using pour plate techniques (Oyeleke and Manga, 2008). The plates were incubated at 37^0C for 24 hours. Visible bacterial colonies were counted and recorded as colony forming units per gram (CFU/g)

The numbers of colonies counted were multiplied by the reciprocal of the dilution factor plated and divided by the volume of inoculums used, to obtain the colony forming unit per millimeter (cfu/ml) of each sample.

Isolation of Pure Culture (sub-culture)

Each typical colony was further sub cultured for more identification; MacConkey, Manitol Salt and EMB agar were prepared and allowed to cool to 45^0C then transferred to sterile petri dishes. Each colony was picked using a sterile wire loop and streaked it into the freshly prepared solidified media and incubated at 37 ℃ for 24 hrs. (Cheesbrough, 2000)

Identification of Bacteria

Identification Bacterial isolates were identified based on colonial morphology, Gram staining reactions and series of biochemical tests.

Gram Staining

A smear of the bacterial colony was made at the center of a grease-free slide and allowed to dry, then heat fixed. The fixed smear was flooded with crystal violet stain for 30 seconds, and then washed off with clean water. The smear was flooded with Lugol's iodine for 30 seconds after which it was washed off with clean water. Acetone was then added and immediately washed off. Neutral red was added for 2 minutes, and then washed off with clean water. Back of the slide was wiped, and then the glass drained and allowed to air dry, after which microscopy was done (Cheesbrough, 2000).

Morphological Characteristics

The identification was achieved by placing a drop of the oil immersion on smeared stained slide with the aid of a dropper. The slide was then mounted and observed with x100 objectives lens respectively. The organisms encountered were identified according to (Cheesbrough, 2000).

Biochemical Test

To identify bacteria, we must rely heavily on biochemical testing. The types of biochemical reactions each organism undergoes act as a "thumbprint" for its identification.

Catalase Test: A drop of hydrogen peroxide was put on a glass slide. With the use of a sterile wire loop, colonies of the test organism were emulsified in the hydrogen peroxide. Catalase positive reaction was seen by their immediate production of bubbles (Cheesbrough, 2000).

Coagulase Test: A drop of normal saline was put on a slide; a colony of the test organism was emulsified in the saline. A drop of human plasma was added and mixed gently. After about 10 second it was observed for cloting. (Cheesbrough, 2000).

Indole Test: The test organism was inoculated into Bijou bottle containing 3ml of sterile tryptone water, incubated at 35-37°C for 48hrs. This was followed by the addition of 0.5ml Kovac's reagent. Red color on the surface layer within 10 minutes indicated positive test for indole. (Cheesbrough, 2000).

Oxidase Test: Filter paper was soaked with 2 drops of freshly prepared oxidase reagent. Colony of the test organism was smeared on the filter paper using glass rod. Positive oxidase was indicted by the production of a deep purple/blue colour within 10 seconds (Cheesbrough, 2000)

Citrate Utilization Test: This test was done by inoculating the organism into Simon's citrate agar slopes which were then incubated at 370C. A change in colour of the medium from green to blue is considered positive (Cheesbrough, 2000)

Urease Production Test: This was carried out by inoculating the urea slopes with colonies of the organism and incubated at 370C for 24 hours. A change in colour of the medium from yellow to pink/red was indicative of a positive result (Cheesbrough, 2000).

MRVP test: To determine the ability of the organisms to produce neutral end product (acetoin) from glucose fermentation.

Procedure

1. Inoculate the tested organism into 2 tubes of MR-VP broth.
2. Incubate the tubes at 37°C for 24 hours
3. After Incubation: Run the MR test in the tube 1, and the VP test in tube 2. – For methyl red: Add 6-8 drops of methyl red reagent. – For Voges-Proskauer: Add 12 drops of Barritt's A (α-naphthol), mix, 4 drops of Barritt's B (40% KOH),

mix – Let sit, for at least 1hour. Red precipitate indicates a positive result. (Cheesbrough, 2000).

Anti-fungi Susceptibility Test

Antifungal susceptibility testing methods are available to detect antifungal resistance and to determine the best treatment for a specific fungus. Clinical microbiology relies on these methods to select the agent of choice for a fungal infection, and to know the local and the global epidemiology of antifungal resistance.

Agar-Based Methods

The agar-based method was carried out using Mueller-Hinton agar. The antifungal drugs used are Ketoconazole, Fluconazole and Greseofulvin The fungi was inoculated by streaking method onto plates containing Mueller-Hinton agar and the paper disks containing the antifungal drug (Ketoconazole, Fluconazole and Greseofulvin) were placed on the inoculated plates and the plates was incubated at 35 ^{o} C/room temperature for 2 days; some strains showed insufficient growth and was left for 3 days of incubation. The results of the susceptibility test according to the zone diameter interpretative criteria for greseofulvin, fluconazole and ketoconazole for fungi species allows to classify the isolate in one of the following categories: susceptible and resistant. After incubation, the zone of inhibition was measured in mm (Ana alastruey-izquierdo., 2009).

RESULTS

The result of these analysis shows that all samples were contaminated with bacteria. The bacteria isolated are *Staphylococcus aureus*, *Staphylococcus spp., Streptococcus spp.*, *Pseudomonas aeruginosa*, *Escherichia coli, Klebsiella spp. and Bacillus spp.*

Table 1 shows the count of the bacteria isolated from fura. The colony count of bacteria Sample 1 shows the highest number of bacteria with the colony count of 7.2 x10^{4} CFU/g and samples 5 shows the lowest number of bacteria with the colony count of 2.7 x10^{4}CFU/g.

Table 2: morphological characteristics of bacteria isolated from locally made fura.

Table 3: shows the different biochemical tests carried out on the gram negative bacteria, to identify the microorganisms include triple sugar iron (TSI), motility test, nitrate test, oxidase test, methyl red, urease test, Voges Proskauer Test, citrate and TSI test.

Table 4: Biochemical test for gram positive bacteria which include Grams reaction, Colony arrangement, Catalase Test and Coagulase test.

Table 5: Total Number of Isolate, *Staphylococcus aureus* and *Streptococcus* spp have the highest number of isolates of which is 7. *Escherichia coli, klebsiella and Bacillus* spp have the least number of isolates which is 3.

Table 6: Shows the antimicrobial susceptibility test pattern of *Pseudomonas aeruginosa*, *Escherichia coli, klebsiella and Bacillus* spp from locally made fura antibiotics used include: CH = chloramphenicol (30ug), CPX = ciprofloxacin (30ug), AU = Augmentin (30ug), PEF = pefloxacin (30ug), OFX = tarivid (10ug), SP = sparfloxacin (10ug)

Table 7: Shows the antimicrobial susceptibility test pattern of *Staphylococcus aureus*, *Staphylococcus spp, Streptococcus spp* from locally made fura antibiotics used include: SXT= Septrin (30ug), R= receptrin (25ug), CPX= Ciprofloxacin (30ug), PEX= Pefloxacin (30ug), E= Erythromycin (10ug), CN= Gentamycin (10ug).

Table 8: Twenty samples of fura were obtained from four different locations which are: Kasua Mata (KM), Hayin Gada (HG), Fudma Gate (FG), and Isa Kaita road (IK).

Out of 20 samples, thirteen samples were found to contain fungi. Fura obtained from Kasua Mata (KM) has the highest fungi count, the least count were found in samples obtained from Hayin Gada(GM), Fudma Gate(FG) and Isa Kaita respectively.

Table 9: *Aspergillus fumigatus* was isolated from five samples (samples 1,3,4,7 and 8). *Aspergillus flavus*, *Aspergillus tereus*, *Aspergillus niger*, and *Aspergillus nidulans* were isolated from one sample each which are samples 2, 6, 11, and 15 respectively. *Candida albicans* was isolated from four samples (samples 12, 13, 14 and 16).

Table 10: Total number of fungi isolates isolated from the fura samples were 13. Total number of positive samples from the fura were13. *Aspergillus fumigatus* and *Candida albican* have the highest number of isolates which are with percentage of 38.5% and 30.7% respectively. *Aspergillus flavus*, *Aspergillus tereus*, *Aspergillus niger*, and *Aspergillus nidulans* have the least number of isolates which is 5%.

Table 11 shows the antifungal susceptibility test of *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus terreus*, *Aspergillus niger*, *Aspergillus nidulans* and *Candida albican*, isolated from locally made fura. The antifungal drugs used are Ketoconazole, Fluconazole and Greseofulvin.

Out of five (5) isolates of Aspergillus fumigates, two (2) was susceptible, one(1) was intermediate and one(1) was resistant to Ketoconazole. Two (2) was susceptible, one (1) was intermediate and two (2) were resistant to Fluconazole. Zero (0) was susceptible, zero (0) was intermediate and five (5) were resistant to Greseofulvin.

The only isolate of Aspergillus tereus was susceptible to Ketoconazole, resistant to Fluconazole and resistant to Greseofulvin.

The only isolate of Aspergillus niger was susceptible to Ketoconazole, susceptible to Fluconazole and susceptible to Greseofulvin.

The only isolate of Aspergillus nidulans was susceptible to Ketoconazole, susceptible to Fluconazole and resistant to Greseofulvin.

Out of four (4) isolates of Candida albicans two (2) were susceptible, zero (0) was intermediate and two (2) were resistant to Ketoconazole. One (1) was susceptible, zero (0) was intermediate and three (3) were resistant to Fluconazole. Zero (0) was susceptible, zero (0) was intermediate and four (4) was resistant to Greseofulvin.

TABLE 1: Total Bacterial Colony Count on Fura Sold in Dutsin-ma

Samples	Colony count (CFU/g)	standard by WHO ($<10^5$)
Sample 1	7.2×10^4	
Sample 2	3.2×10^4	
Sample 3	3.4×10^4	
Sample 4	4.2×10^4	
Sample 5	2.7×10^4	
Sample 6	5.6×10^4	
Sample 7	6.4×10^4	
Sample 8	3.6×10^4	
Sample 9	4.2×10^4	
Sample 10	5.6×10^4	
Sample 12	6.1×10^4	
Sample 13	4.0×10^4	
Sample 14	2.9×10^4	
Sample 15	3.3×10^4	
Sample 16	4.1×10^4	
Sample 17	5.0×10^4	
Sample 18	5.4×10^4	
Sample 19	4.4×10^4	
Sample 20	5.5×10^4	

TABLE 2: Colonial and Morphological Characteristics of Bacteria Isolated from Fura

MEDIA	MORPHOLOGY	BACTERIA
NA	Greenish coloration, Large, opaque, flat colonies with fruity odour	*Pseudomonas spp*
NA	Circular rough colony, opaque, slightly yellow with jagged edge	*Bacillus spp*
NA	Round, smooth, convex, with a glistening edge	*Staphylococcus spp*
NA	Round, convex with sharp edge	*Staphylococcus aureus*
MA	Large, mucoid sticky and pink in colour	*Klesiella spp*
MA	Pink, dry and donut-shaped, surrounded by a dark pink area	*E.coli*
NA	Gray to yellow with a glistening edge	*Streptococcus spp*

Keys: NA = nutrient agar MA = macConkey agar

TABLE 3: Biochemical Test for Gram Negative Bacteria

S/N	Sample	Indole Test	Methly Red Test	Voges Proskauer Test	Citrate Test	Urease Test	Nitrate Test	Oxidase Test	Motility Test	Organisms
1	S6	-	-	-	+	-	+	+	+	*Pseudomonas aeruginosa*
2	S8	-	-	-	+	-	+	+	+	*Pseudomonas aeruginosa*
3	S10	-	-	-	+	-	+	+	+	*Pseudomonas aeruginosa*
4	S19	-	-	-	+	-	+	+	+	*Pseudomonas aeruginosa*
5	S15	-	-	+	+	+	+	-	-	*Klebsiella spp*
6	S18	-	-	+	+	+	+	-	-	*Klebsiella spp*
7	S20	-	-	+	+	+	+	-	-	*Klebsiella spp*
8	S16	+	+	-	-	-	+	-	+	*Escherichia coli*
9	S17	+	+	-	-	-	+	-	+	*Escherichia coli*
10	S14	+	+	-	-	-	+	-	+	*Escherichia coli*
11	S2	-	-	+	+	-	+	-	+	*Bacillus spp*
12	S13	-	-	+	+	-	+	-	+	*Bacillus spp*
13	S5	-	-	+	+	-	+	-	+	*Bacillus spp*

Keys: + = positive, - =negative, S= sample

TABLE 4: Biochemical Test for Gram Positive Bacteria

S/N	Sample	Grams Reaction	Colony arrangement	Catalase Test	Coagulase test	Organisms
1	S3	+	Cluster	+	-	*Staphylococcus spp*
2	S7	+	Cluster	+	-	*Staphylococcus spp*
3	S9	+	Cluster	+	-	*Staphylococcus spp*
4	S17	+	Cluster	+	-	*Staphylococcus spp*
5	S14	+	Cluster	+	-	*Staphylococcus spp*
6	S1	+	Cluster	+	+	*Staphylococcus aureus*
7	S11	+	Cluster	+	+	*Staphylococcus aureus*
8	S4	+	Cluster	+	+	*Staphylococcus aureus*
9	S8	+	Cluster	+	+	*Staphylococcus aureus*
10	S9	+	Cluster	+	+	*Staphylococcus aureus*
11	S12	+	Cluster	+	+	*Staphylococcus aureus*
12	S15	+	Chain	-	-	*Streptococcus spp*
13	S16	+	Chain	-	-	*Streptococcus spp*
14	S12	+	Chain	-	-	*Streptococcus spp*
15	S4	+	Chain	-	-	*Streptococcus spp*
16	S19	+	Chain	-	-	*Streptococcus spp*
17	S8	+	Chain	-	-	*Streptococcus spp*
18	S11	+	Chain	-	-	*Streptococcus spp*
19	S18	+	Chain	-	-	*Streptococcus spp*

Keys: + = positive, - =negative, S= sample Key: S= sample

Table 5: Occurrence frequency of bacteria isolates

ISOLATED BACTERIA	NUMBER OF ISOLATE	FREQUENCY (%)
Pseudomonas aeruginosa	5	14.7
Klebsiella sp	3	8.8
Escherichia coli	3	8.8
Bacillus spp	3	8.8
Staphylococcus spp	6	17.6
Staphylococcus aureus	7	20.6
Streptococcus sp	7	20.6
TOTAL	**34**	**100**

TABLE 6: The Susceptibility Test of *Pseudomonas aeruginosa*, *Escherichia Coli and Klebsiella*

S/N	Organisms	No. of organisms	CH (30ug)			SP (10ug)			CPX (30ug)			PEF (30ug)			OFX (10ug)			AU (30ug)		
1	*Psuedomonas aeruginosa*	5	S 1	I 2	R 2	S 2	I 2	R 1	S 4	I 1	R 0	S 1	I 2	R 2	S 1	I 2	R 2	S 2	I 2	R 1
2	*E. coli*	3	1	2	0	1	1	1	2	1	0	1	2	0	1	1	1	1	1	1
3	*Klebsiella spp*	3	2	1	0	1	2	0	3	0	0	1	2	0	0	1	1	2	1	0
4	*Basillus spp*	3	0	1	2	O	3	1	2	1	0	2	1	0	2	1	0	0	2	1

Key: S= sensitive, I= intermediate, R = resistant, CH = chloramphenicol (30ug), CPX = ciprofloxacin (30ug), AU = Augmentin (30ug), PEF = pefloxacin (30ug), OFX = tarivid (10ug), SP = sparfloxacin (10ug)

TABLE 7: **The susceptibility of Staphylococcus spp, Staphylococcus aureus *and* Streptococcus spp**

S/N	Organisms	No. of organism	SXT (30ug)			R (25ug)			CPX (30ug)			PEF (30ug)			E (10ug)			CN (10ug)		
1	*Staphylococcus spp*	6	S 1	I 4	R 1	S 1	I 3	R 2	S 3	I 2	R 1	S 4	I 2	R 0	S 3	I 2	R 1	S 5	I 1	R 0
2	*Staphylococcus aureus*	7	4	2	1	2	3	2	5	1	1	6	1	0	3	2	2	6	1	0
3	*Streptococcus spp*	7	2	1	4	2	3	2	5	1	1	4	2	1	2	2	3	4	2	1

Keys: S= susceptible, I= intermediate and R= resistance, SXT= Septrin (30ug), R= receptrin (25ug), CPX= Ciprofloxacin (30ug), PEX= Pefloxacin (30ug), E= Erythromycin (10ug), CN= Gentamycin (10ug). Range of AST according to clinical laboratory standard institute (CLSI) 2020
≥ 20mm is susceptible, 14-19mm intermediate and below 14mm is resistance.

Table 8: Total Fungal Count of the Fura Samples

SAMPLES	LOCATIONS	COLONY COUNT(CFU/ml)
1	HG 1	$1x10^3$
2	HG 2	$1x10^3$
3	HG 3	$1x10^3$
4	HG 4	$1x10^3$
5	HG 5	No Count
6	FG 6	$1x10^3$
7	FG 7	$1x10^3$
8	FG 8	$1x10^3$
9	FG 9	No Count
10	FG 10	No Count
11	KM 11	$1x10^3$
12	KM 12	$4x10^3$
13	KM 13	$3x10^3$
14	KM 14	$5x10^3$
15	KM 15	$1x10^3$
16	IK 16	$3x10^3$
17	IK 17	No Count
18	IK 18	No Count
19	IK 19	No Count
20	IK 20	No Count

KEY: HG= Hayin Gada, FG=Fudma Gate, KM=Kasua Mata, IK=Isa Kaita

Table 9: Cultural, Morphological and Fungi Isolated from The fura Samples

SAMPLES	LOCATION	CULTURE	MICROSCOPIC MORPHOLOGY	ISOLATED FUNGI
1	HG 1	Blue-green	Conidia head	*Aspergillus fumigates*
2	HG 2	Yellow-green	Conidia head, rough walled near vesicle	*Aspergillus flavus*
3	HG 3	Blue-green	Conidia head	*Aspergillus fumigates*
4	HG 4	Blue-green	Conidia head	*Aspergillus fumigates*
5	HG 5	No Growth	No Growth	*No Growth*
6	FG 6	Cinnamon to brown	Conidia head(are biserate)	*Aspergillus tereus*
7	FG 7	Blue-green	Conidia head	*Aspergillus fumigates*
8	FG 8	Blue-green	Conidia head	*Aspergillus fumigates*
9	FG 9	No Growth	No Growth	No Growth
10	FG 10	No Growth	No Growth	No Growth
11	KM 11	Black	Conidia head	*Aspergillus niger*
12	KM 12	White	Oval	*Candida albicans*
13	KM 13	White	Oval	*Candida albicans*
14	KM 14	White		*Candida albicans*
15	KM 15	Purplish red to olive	Conidia head	*Aspergillus nidulans*
16	IK 16	White	Oval	*Candida albicans*
17	IK 17	No Growth	No Growth	No Growth
18	IK 18	No Growth	No Growth	No Growth
19	IK 19	No Growth	No Growth	No Growth
20	IK 20	No Growth	No Growth	No Growth

KEY: HG= Hayin Gada, FG=Fudma Gate, KM=Kasua Mata, IK=Isa Kaita

Table 10: Frequency of Fungal isolates from Fura samples

ISOLATED FUNGI	NO. OF POSITIVE SAMPLES	FREQUENCY (%)
Aspergillus fumigates	5	38.5
Aspergillus flavus	1	7.7
Aspergillus tereus	1	7.7
Aspergillus niger	1	7.7
Aspergillus nidulans	1	7.7
Candida albicans	4	30.7
TOTAL	13	100

Table 11: Antifungal Susceptibility Test For The Fungi Isolated From The Fura Sample

FUNGI ISOLATED	NO. OF ISOLATE	KETOCONAZOLE			FLUCONAZOLE			GRESEOFULVIN		
		S	I	R	S	I	R	S	I	R
Aspergillus flavus	1	1	0	0	0	1	0	0	0	1
Aspergillus fumigatus	5	2	1	1	2	1	2	0	0	5
Aspergillus tereus	1	1	0	0	0	0	1	0	0	1
Aspergillus niger	1	1	0	0	1	0	0	1	0	0
Aspergillus nidulans	1	1	0	0	1	0	0	0	0	1
Candida albicans	4	2	0	2	1	0	3	0	0	4

KEY: S=Susceptible, I=Intermediate, R=Resistant.
< 14mm= Resistant, 15mm-19mm= Intermediate, 20mm and above= Susceptible.

DISCUSSION

The result of these analyses shows that all samples were contaminated with bacteria, which are potential source of food borne infection and some related diseases to the consumers of this product in the sampling areas. The bacteria isolated include *Staphylococcus aureus* (17.6%), *Staphylococcus spp* (20.6%)*, Streptococcus spp* (20.6%), *Pseudomonas aeruginosa* (14.7%), *Escherichia coli* (8.8%)*, Klebsiella spp* (8.8%) *and Bacillus spp* (8.8%). There were many reports on isolation and identification of bacteria from locally made fura from Federal University Wukari (Owusu-Kwarteng *et al.,* 2010) worked on bacterial isolated from locally made fura reported *S. aureus* as the predominant organisms accounting for 35.8% followed by *Staphylococcus spp* 34%, *Streptococcus spp* 32%, *P. aeruginosa* 21.8%, *E. coli* 15.3% *Klebsiella spp* 14.2*%*, *Bacillus spp* 13.9%.This preponderance of *S. aureus* is in agreement with other study, this may be due to the organisms been part of the normal flora of the skin and can easily contaminate locally made fura. According to the present study, the most common bacterial isolated were *Staphylococcus spp* (20.6%)*, Streptococcus spp* (20.6%). In other study, the most common bacterial where *S. aureus.* (Okereke *et al.,* 2004). Another report on isolation and identification of bacteria from locally made fura from University of Ghana, Department of Nutrition and Food Science (Jideani *et al.,* 2001) The isolated organisms were identified based on morphological, physiological and biochemical characteristics were *Streptococcus spp.* (14.3%), *Streptococcus spp* (8.5%), *Klebsiella spp* (5%), *P. aeruginosa* (4.3%) *Bacillus spp* (3.5%). This might be due to the fact that the fura production was conducted under unhygienic condition. This might contaminate the fura aerially as a result of suspension of the spores of these organisms in the air. From the antimicrobial susceptibility test Out of the six antibiotics (Ciprofloxacin, Chloramphenicol, Augmentin, Pefloxacin, Tarivid and Sparfloxacin) used to carry out the susceptibility test; the isolated gram-negative bacteria were more susceptible to Ciprofloxacin and more resistant to Tarivid.

The result obtained for fungal isolation and identification shows that there is presence of pathogenic fungi that may be potential source of food borne infection and some related diseases to the consumers of this product in the sampling areas. This might be due to the fact that the fura production was conducted under unhygienic condition. The colony forming unit of fungi ranging from 1×10^3 to $5x10^3$ Cfu/ml which also signifies high contamination that might be due to the suspended spores of the organisms in the air that could find their way into the fura during processing and selling. This did not coincide with the work of Adebesin *et al*. (2001) with fungal colony count ranging from $1.0x10^4$ to $2.\ 9x10^4$ cfu/g, the dilution factors differs (10^{-2} and 10^{-6}), if the spores of these organisms in the air which is in line with the finding of Adebesin *et al*., (2001) and Owusu-Kwarteng *et al*. (2010). The fungi with high frequency of occurrence was *Aspergillus fumigatus* of about 38.5% followed by 30.7% *Candida albican*, which might be as a result of their spores were prevalent and easily aerosolized, this is in line with the finding of Adebesin *et al*. (2001) and Owusu-Kwarteng *et al*. (2010). Then *Aspergillus flavus*, *Apergillus niger*, *Aspergillus terreus* and *Aspergillus nidulans* are the only fungi with least percentage of occurrence 7.7%, this might be attributed to the fact that the organisms being rarely found in the air, this is in line with the findings of Owusu-Kwarteng *et al.* (2010).The fungi isolated were *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus nidulans*, *Aspergillus terreus*, *Aspergillus fumigatus*, *Candida albican*. This might contaminate the fura aerially as a result of suspension. Out of the three antifungal drugs (Ketocanazole, Fluconazole and Greseofulvin) used to carry out the susceptibility test, the isolated fungi were more susceptible to Ketoconazole and more resistant to Greseofulvin.

CONCLUSION

The result of these analyses shows that all samples the total bacterial counts for all samples were within the acceptable limit by WHO of greater or equal to 10^5 and contaminated with bacteria, which are potential source of food borne infection and some related diseases to the consumers of this product in the sampling areas. The bacteria isolated include *Staphylococcus aureus* (17.6%), *Staphylococcus* spp (20.6%)*, Streptococcus spp* (20.6%), *Pseudomonas*

aeruginosa (14.7%), *Escherichia coli* (8.8%), From the antimicrobial susceptibility test out of the six antibiotics (Ciprofloxacin, Chloramphenicol, Augmentin, Pefloxacin, Tarivid and Sparfloxacin) the isolated gram negative bacteria were more susceptible to Ciprofloxacin and more resistant to Tarivid of *Staphylococcus aureus*, *Staphylococcus spp, Streptococcus spp* from locally made fura antibiotics used include: SXT= Septrin (30ug), R= receptrin (25ug), CPX= Ciprofloxacin (30ug), PEX= Pefloxacin (30ug), E= Erythromycin (10ug), CN= Gentamycin (10ug) respectively.

The result of these analyses shows that all samples the total fungal counts for all samples were within the acceptable limit by WHO of greater or equal to 10^5 Total number of fungi isolates isolated from the fura samples were 13. Total number of positive samples from the fura were13. *Aspergillus fumigatus* and *Candida albican* have the highest number of isolates which are with percentage of 38.5% and 30.7% respectively. *Aspergillus flavus*, *Aspergillus tereus*, *Aspergillus niger*, and *Aspergillus nidulans* have the least number of isolates which is 5%.

The antifungal susceptibility test of *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus terreus*, *Aspergillus niger*, *Aspergillus nidulans* and *Candida albican*, isolated from locally made fura. The antifungal drugs used are Ketoconazole, Fluconazole and Greseofulvin.

Out of five (5) isolates of *Aspergillus fumigatus*, two *Klebsiella* spp (8.8%) *and Bacillus* spp (8.8%). (2) was susceptible, one (1) was intermediate and one (1) was resistant to Ketoconazole. Two (2) was susceptible, one (1) was intermediate and two (2) were resistant to Fluconazole. Zero (0) was susceptible, zero (0) was intermediate and five (5) were resistant to Greseofulvin.

The only isolate of *Aspergillus tereus* was susceptible to Ketoconazole, resistant to Fluconazole and resistant to Greseofulvin.

RECOMMENDATIONS

Fura serve as one of the sources of beverages for the inhabitants of Dutsinma, it is recommended that microbiological examination of this food be carried out periodically so as to assess their suitability for consumption. However, some number of measures can be taken to minimize fura contamination which may include:

1. The producer of Fura should be more educated as to understand the basic concept of hygiene; the materials used for processing and preparing of fura should be sterilized.
2. Processing and packaging of fura should be carried out under hygienic environment to avoid contamination.
3. Adequate heating process should be employed where applicable.

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