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Research Article

Microbiological Assessment of Selected Groundnut Based Snack "Kulikuli" and "Donkwa" in Ogbomoso And Ilorin, Nigeria

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Abstract

In this study, the microbial quality assessments of two peanut based snacks were analyzed. Samples were obtained from 'Orita Naira' market, Ogbomosho, Oyo State and 'Oja Titun' Ilorin, Kwara State, Nigeria. The controls were prepared under hygienic condition in the Food processing laboratory, Ladoke Akintola University of Technology, Ogbomoso. These samples were analyzed to investigate their safety. Thus, microbial colony estimation and characterization were carried out; these include microbial load, frequency of occurrence and antimicrobial sensitivity test. All the samples had bacterial and fungal contamination at varying degree; microbial load in terms of percentage frequency of occurrence of bacteria was between 5 - 30%, the percentage frequency of occurrence for fungi was 5 - 16%. For bacteria, the highest percentage microbial count was *Salmonella* spp (30%) while the least was *E. coli* (5%), while for fungi, the highest and lowest percentage microbial count were 16 and 5% respectively. Total Viable Count ranged from 1.3 ± 0.10 x 103 to 4.10 ± 0.10 x 103 (cfu/ml), coliform ranged between 0.00-5.40 ± 0.40 cfu/ml, while mould count ranged from 1.00 ± 0.00 - 3.87 ± 0.31. Sensitivity test showed that bacteria inhibition zone of 32 mm and 25 mm in Kulikuli and Donkwa respectively, while fungal sensitivity test indicated 26 mm, 32 mm in kulikuli and donkwa respectively. With 30% *salmonella* spp found in kulikuli and 10% *E. coli* found in donkwa, it shows poor hygiene in processing and handling.

Keywords: Isolation; Hygiene; Contamination; Processing; Organism

Introduction

Kulikuli and Donkwa, are groundnut-based snacks indigenous to the West African coasts. Being a snack, it is consumed by all age range but more specifically by school-age children and the middle age. It is also used as a major ingredient in poultry feed formulation [1]. The snacks are usually produced from groundnut during oil extraction from the groundnut. These snacks are simply regarded as the fried residue obtained from the process of local vegetable oil extraction from groundnut [2]. Kulikuli is a peanut based while Donkwa is a maize-peanut based. The two snacks have been reported to be rich in protein and crude fat reflecting the composition of groundnut [3,4]. However, Aletor and Ojelabi [3] reported that these snacks could serve as a major protein supplement since it contained high crude protein.

The enterobacteria are a large group of related bacteria that are capable of food and water contamination through faecal sources. Many of the strains and species are known to be enterotoxicgenic and contribute majorly to cases such as diarrheal illnesses experienced by man [5,6]. Therefore, in the bid to enhance human health and secure food safety as well as public health enlightenment on food-borne illnesses, there is a need to evaluate the processing, handling effect on the microbial load of these snack available for human consumption in markets across Nigeria [7,8] Also, considering the fact that information obtained via questionnaire indicates that many school-age children consume this product that is known to be locally processed, there is an urgent need to ascertain the cause of the frequent diarrhea cases reported by patients of this age group with history of contact with this food [1,9]. In essence, this research aims at evaluating Kulikuli samples obtained from two states in Nigeria for microbial contamination and proximate changes.

Materials and Methods

Sample collection

The material used for this research work were purchased at Orita Naira, Ogbomosho, Oyo state and Oja tuntun in Ilorin Kwara

State. The control was prepared under clean condition in the Food Science and engineering department laboratory, Ladoke Akintola University of Technology Ogbomoso Oyo State, Nigeria.

Methods

The samples used for this work were aseptically taken from the selling points. Proximate analysis was done according to AOAC [10], Microbial Analysis by method described by Fawole and Oso (2004). Isolation and identification of Microorganism were done by standard microbiological methods. Characterization of bacteria isolates by Fawole and Oso (2004). Mycological analysis of samples was carried out according to method described by Samson., et al. [11]. The antimicrobial sensitivity test of the isolates was carried out using the disc agar diffusion methods of CM-12-8PR100 and CM-12-8NR100.

Culture media

The media used for the microbiological isolation of microorganism were nutrient agar and potato dextrose agar. Nutrient agar was used for the isolation of bacteria and potato dextrose agar used for the isolation of fungi. The media were prepared according to the manufacturer's instruction. The isolation of the organisms, culture examination, culture preservation, gram staining and other biochemical test were carried out by standard microbiological methods.

Results and Discussion

The organism with highest number of occurrence among the isolates in kulikuli was *Salmonella spp with 30%,* while *E. coli* and *Pseudomonas aureginosa* were the least with 5% each as shown in Table 1. In donkua, the highest were *Staph. Aureus and Bacillus spp* with 15% each while *Micrococcus spp, Pseudomonas aureginosa* and *E. coli* have the same value of 10%. The frequency of occurrence of *E. Coli* isolated from Kulikuli and Donkwa are (5%) and 10%, respectively, while *Salmonella* spp has a value of 30% in kulikuli but none was found in donkwa. Proteus was 10% in kulikuli and nil in donkwa; *Klebsiella* (15%) kulikuli and nil in donkwa, *Pseudomonas aureginosa* have values of 5% and 10% kulikuli and donkwa, respectively, *Staphylococcus aureus* was 15% in both kulikuli and donkwa. *Bacillus* spp was 15% in donkwa and nil in kulikuli. *Micrococcus* spp value was 10% in donkwa but nil in kulikuli. The frequency of occurrence of fungi isolated according to Table 2 are; *Aspergillus spp.* (16%) in kulikuli, none in donkwa, *Aspergillus niger* nil in kulikuli but 16% in donkwa, *Aspergillus flavus* was has nil value in kulikuli but 11% in donkwa; *Fusarium* spp has 5% value in kulikuli but nil in donkwa, *Penicillum* spp (11%) in kulikuli and nil in donkwa, *Penicilum citrinum* (11%) both in kulikuli and donkwa, *Rhizopus stolonifer* (11%) only in donkwa but in kulikuli, *Rhizopus* spp; (11%) only in kulikuli and nil in donkwa, *Trichaderma* spp (5%) only in kulikuli and nil in donkwa; *Mucor* spp (5%) in donkwa but nil kulikuli.

Bacteria isolated	Kulikuli		Donkwa	
	Frequency	%	Frequency	%
E. Coli	1	5	2	10
Salmonella spp	3	30	-	-
Proteus spp.	2	10	-	-
Klebsiella spp.	3	15	-	-
Pseudomoninas aureginosa	1	5	2	10
Staphylococcus aureus	3	15	3	15
Bacillus spp.	3	-	3	15
Micrococcus spp.	2	-	2	10

Table 1: Average frequency of occurrence of bacteria isolated from Kulikuli and Donkwa.

Organisms	Kulikuli		Donkwa	
	Frequency	(%)	Frequency	(%)
Aspergillus spp	3	16		-
A. niger	-	-	3	16
A. flavus	-	-	2	11
Fusarium spp	1	5	-	-
Penicillum spp	2	11	2	-
Penicillum citrinum	2	11	2	11
Rhizopus stolonifer	-	-	2	11
Rhizopus spp.	2	11	-	-
Trichadema spp.	1	5	-	-
Mucor spp.	-	-	1	5

Table 2: Average frequency of occurrence of fungi isolated from Kulikuli and Donkwa.

-; Not detected.

Among the samples taken according to Table 3, Donkwa sample F has the highest load of coliform growth of 5.40 ± 0.40 x 10^3 cfu/ml, while sample C (Kulikuli) has the highest mould count of 3.87 ± 0.31 x 10^3 cfu/ml. TVC of 4.10 ± 0.10 x 10^3 was observed in sample C (kulikuli). These results are within the range obtained in literature [1,9].

As shown in Table 3; both the fungal and bacteria counts were found to be between the range of 10^3cfu/ml and 10^5cfu/ml respectively. As recommended by international commission of microbiological specification for food [12], these two products were out of range of standard for type A class of food, but within the limit set by the international commission on microbiological specification of food for class B, category 2; hence, the snacks fall in to acceptable category but it is expedient that the processing and handling method must be reviewed to ensure improvement toward hygienic condition during preparation, packaging and selling of these two products.

Sample	TVC10^3 cfu/ml	Coliform 10^3 cfu/ml	Mould Count 10^5 cfu/ml
A	1.30 ± 0.10^a	0.00± 0.00^a	1.00 ± 0.00^a
B	2.47 ± 0.25^c	3.20± 0.40^b	2.00 ± 0.10^b
C	4.10 ± 0.10^d	0.00 ± 0.00^a	3.87 ± 0.31^d
D	1.40 ± 0.20^a	0.00 ± 0.00^a	1.00 ± 0.10^a
E	2.00 ± 0.20^b	4.80 ± 0.60^c	3.00 ± 0.30^c
F	2.40 ± 0.40^b	5.40 ± 0.40^d	2.40 ± 0.40^b

Table 3: Colony Count for Kulikuli and Donkwa (Microbial analysis).

Sample A: Kulikuli Control; Samples B: Kulikuli Orita Naira Market; and Sample C: Kulikuli (Oja Tuntun Ilorin market); Sample D: Donkwa control; Samples E: Donkwa Orita Naira Market; and Sample F: Donkwa Oja Tuntun Ilorin Market.

Antimicrobial sensitivity test carried out; the bacteria test for Kulikuli has the highest zone of 32 mm against *Salmonella* by Gentamycin while the list zone was observed in Ampicillin against *E. coli.* It was also observed that Gentamycin proved most effective against *E. coli, Proteus, Klebsiella and Pseudomonas* except *Staphylococcus* where Ampicillin proved most effective through the highest value of zone of inhibition as shown in table 4. Fungal sensitivity test for kulikuli according to table 5 shows the highest zone 26 mm in Nystatin against *Rhizopus spp.,* while the least zone of 13 mm was observed in Griseofulin against *A. niger,* and *Trchoderma.*

Bacteria sensitivity test in donkwa has the highest zone of 20 mm in Angumentin against *Micrococcus spp* while the least zone of 11 mm was in Agumentin for *Staphylococcus aureus* and 11 mm for Micrococcus spp in *Streptomycin* as shown in Table 6. How-

Antibiotic	Zone of inhibition in mm					
	E. coli	*Salmonella*	*Proteus*	*Klebsiella*	*Pseudomonas*	*Staphylococcus*
Chloramphenicol	-	-	-	-	-	-
Amoxicillin	16	12	13	14	15	6
Augmentin	5	18	12	15	6	7
Gentamycin	26	32	28	25	21	17
Streptomycin	23	12	14	16	17	12
Ampicillin	11	12	15	18	19	20
Erythromycin	-	-	-	-	-	-
Septrin	-	-	-	-	-	-
Pefloxacin	-	-	-	-	-	-

Table 4: Bacterial sensitivity test of Kulikuli samples isolates.

-: Not detected.

Anti fungicide	Zone of inhibition in mm				
	Aspergillus spp.	*Fusarium spp.*	*Penicillium spp.*	*Rhizopus spp.*	*Trichoderma spp.*
Nystatin	18	21	24	26	14
Griseofulin	13	20	18	14	13
Ketxonazole	-	-	-	-	-
Mycoten	-	-	-	-	-

Table 5: Fungi sensitivity test of isolates from Kulikuli samples.

-: Not detected.

Antibiotics	Zone of inhibition (mm)			
	Micrococcus spp.	*Bacillus cereus*	*Staphylococcus aureus*	*Pseudomonas aureginosa*
Chloramphenicol	14	17	18	20
Amoxicillin	-	-	-	-
Augmentin	20	19	11	13
Gentamycin	25	17	24	18
Streptomycin	11	08	10	07
Ampicillin	12	15	12	15
Erythromycin	16	12	21	09
Septrin	-	-	-	-
Pefloxacin	-	-	-	-

Table 6: Bacterial sensitivity test of isolates from Donkwa.

-: Not detected.

ever, gentamicin was the most effective against *Micrococcus spp.* and *Staphylococcus aureus* but chloramphenicol was most effect on *Pseudomonas aureginosa* with inhibition diameter of 20 mm as shown in table 6. Table 7 shows Donkwa sensitivity test with highest zone of 32 mm to *Mucor spp* by Nystatin, while the least resistance was *A. niger with* 12 mm zone of inhibition.

Cases of food borne infections sometimes are prevalent with ready-to-eat food. The presence of indicating organism of feacal contamination at 10% level in donkwa shows poor hygiene. With the presence of *Salmonella spp* with 30% value; eating such may be an agent of food infection to the consumers. *Bacillus cereus* can cause gastro-enteritis; an exo-enterotoxin as a result of cell lysis

Fungi	**Zone of Inhibition (mm)**						
	Mucor	***Mucor spp.***	***Rhizopus spp***	***Penicillium citrium***	***Aspergillus flavus***	***Aspergillus Niger***	***Aspergillus tamari***
Nystatin	21	32	24	26	20	12	25
Griseofulin	14	18	16	22	18	14	20
Ketxonazole	-	-	-	-	-	-	-
Myxoten	-	-	-	-	-	-	-

Table 7: Fungal sensitivity test of isolates from Donkwa samples.

-: Not detected.

of *Bacillus cereus* in the intestinal tract. Hence, high percentage of such in food at 15% contamination level is a warning signal to low quality food as reported by Frazier and Westhoff [13].

Conclusion

Microbiological quality of any food determines safety and acceptability by the level and load of microorganisms, the type of organisms isolated. The result of the investigation under study showed that the microbial counts were out of range of standard for type A class of food, but within the limit set by the international commission on microbiological specification of food for class B, category 2. There is need for further effort to improve the handling and hygiene practice of the producer of the snack [12,14-17]. Local processors should be enlightened on hygienic methods of preservation and storage of the kulikulis and donkwa. Furthermore, relevant quality control units must be reactivated to assess the quality of the groundnut kernels from which the kulikulis and other products are made.

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