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Original Article

MICROBIOLOGICAL QUALITY OF TIGERNUT SOLD IN ABAKALIKI, EBONYI STATE

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ABSTRACT

Background of study: This study evaluated the microbiological quality of tiger nuts sold at five locations in Abakaliki: Presco Campus Gate, Ahia Ofu, Ishieke Market, Ogbehausa, and the International Market.

Methodology
A total of 25 tiger nut samples were collected from vendors at each of the different locations, and analyzed for microbial presence and load and pathogens.

Results
The results revealed significant contamination, indicating poor microbiological quality. Pathogens isolated included *Bacillus subtilis*, *Staphylococcus aureus*, *Aspergillus flavus*, *A. niger*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Enterococcus* spp., and *Escherichia coli*. These pathogens pose a risk of foodborne illnesses. Antimicrobial susceptibility testing showed varying resistance levels among pathogens. *Pseudomonas aeruginosa* was most susceptible to Gentamicin (68%) and highly resistant to Amoxicillin (18%). *Bacillus spp.* showed the highest susceptibility to Clindamycin (76%) and resistance to Levofloxacin and Ceftriaxone (9%). *K. pneumoniae* was most susceptible to Azithromycin (66%) and resistant to Clindamycin (13%). *S. typhi* had high susceptibility to Ciprofloxacin (74%) and resistance to Linezolid (26%). *S. aureus* was highly resistant to Meropenem (3%) but sensitive to Vancomycin (80%). *Enterococcus* and *E. coli* showed susceptibility to Amoxicillin and Gentamicin but significant resistance to other antibiotics.

Conclusion
The presence of these pathogens in the tigernuts highlights the need for proper hygienic practices during cultivation, processing, and handling. Strict food safety measures and awareness campaigns are highly essential to minimize risks and ensure consumer safety. These findings underscore the importance of ongoing monitoring to improve the microbiological quality of tiger nuts in Abakaliki.

Keywords: Tigernuts, Microbiological quality, Food safety, Antimicrobial susceptibility, vendors.

INTRODUCTION

Tiger nut, (*Cyperus esculentus Lativum*), also called chufa, yellow nutsedge, earth almond, and ground almond. In Nigeria, for instance, it is called "aya" in Hausa, "aki awusa" in Igbo, "ofio" in Yoruba, and "isipaccara" in Effik. It is a member of the *Cyperaceae* family. It is a perennial crop widely grown throughout Asia, East Africa, parts of Europe, especially in Spain, and the Arabian Peninsula (Abdelkader *et al.,* 2017). The tiger nut is a primary example of early adaptation; its dry tubers were discovered in predynastic tombs dating back approximately 6000 years, and it was a staple food in Egypt where the tubers were used as sweetmeat (Zohary, 1986). There are three types of tiger nut tubers: yellow, brown, and black. While the black variety is not commonly found in Nigeria, it can be obtained in Ghana (Asante *et al.,* 2014). These edible tubers have a sweet, nutty flavour and are rich in protein, carbohydrates, sugars, oil, and fibre (Gambo, and Da'u, 2014).

Recent research has suggested that tiger nuts may have a range of health benefits, including improving digestion, reducing the risk of heart disease, and managing blood sugar levels. Tous *et al.* (2021), stated that tiger nuts contain high levels of dietary fibre, which can help to promote healthy digestion and prevent constipation. Tiger nut contains a substantial amount of lipids (22.14 – 44.92%), with a lipid profile similar to olive oil, and is considered the most suitable lipid for human consumption (Touria *et al*, 2022).

In addition, tiger nuts are a rich source of antioxidants, which can help to reduce inflammation in the body and protect against chronic diseases such as heart disease and cancer (Akinyele, and Oke, 2020). Despite these potential health benefits of Tiger nuts, it has remained a relatively understudied food source. Recently, there has been an increasing interest in the use of tiger nuts for beverage production due to their high nutritional value and potential health benefits.

This research is aimed at providing a comprehensive evaluation of the microbiological quality of ready-to-eat tiger nut tubers sold in the metropolis of Abakaliki. Several factors predispose these products to microbial contamination, such as environmental conditions, and unhealthy processing and harvesting practices. It is difficult to attest to the hygiene practices of these vendors and the tiger nuts are exposed to the environment without proper covering to protect them from dust and other environmental sources of contaminants (Maduka, and Ire, 2018). Bacteriological contamination of tiger nut is common and usually ingested with the contaminants and it's attending consequences, this has led to food poisoning among consumers.

The implicated organisms include *Staphylococcus species, Salmonella species, Enterobacter species, Bacillus cereus, Clostridium species, Acinetobacter species, Corynebacterium species, Neisseria species,* and *Aeromonas specie* (Nnabuike, *et al*, 2022). These bacterial pathogens and contaminants are easily acquired in the cause of harvesting, washing, and drying the tiger nut before selling. These pathogens have contributed to significant public concern as most consumers and vendors are ignorant of the potential health impact. This study tends to isolate, identify, and characterize the antibiotic susceptibility profile of these pathogens present in tiger nuts sold within Abakaliki town.

METHODOLOGY

Sample collection:

The dry and fresh yellow tiger nuts were sourced from five different locations and markets (Presco Campus gate, Ahia ofu, Ishieke market, Ogbehausa, and International market) randomly in March 2023 in Abakaliki Ebonyi State, Nigeria. Five different tiger nuts samples of about 15g each (wet and dry) were purchased from different vendors, packaged in a sterile Ziploc bag, and transported immediately to the laboratory for processing.

Sample processing

The yellow tiger nuts both the wet and dry samples purchased from different vendors at each location were labelled, assigned the name of the purchase point, and numbered 1-5. This gives exactly five samples from each location. The tiger nuts were soaked in 30 ml of sterile normal saline, in sterile bottles for 30 minutes. After the 30mintues the tiger nuts were washed by rocking the bottles strongly. The saline was then transferred into a sterile beaker. The process was repeated for five additional washes and all the washes combined. Aliquots of

the combined saline washes were dispensed into centrifuge tubes and centrifuged at 2500rpm for 5 minutes. The supernatant was decanted and the sediments were re-suspended in 1 ml of saline for microbial examination. The processes were repeated for each group of the samples collected. A ten-fold serial dilution was carried out using the appropriate dilution tubes and pipette, by transferring 1 ml of each sample of tiger nut aliquot into a test tube containing 9 ml of peptone water using a sterile Pasteur pipette and mixing to obtain dilution 10-1 dilutions.

Characterization and Identification of Isolates:

The following media were utilized for the identification of the Isolates, MacConkey agar, mannitol salt agar, Salmonella-Shigella agar, Sheep Blood Agar, and Chocolate agar. All media used were prepared according to the manufacturer's instructions and the materials were sterilized by autoclaving. The 0.1 ml aliquot of the wash of tiger nut from each group (10-1 dilutions) was inoculated aseptically into distinct culture plates and the media was added using the pour plate method. The inoculated petri dishes are incubated by placing them into the incubated with a temperature set at 37^0c for 18-24hrs respectively. As a control, an equal volume (0.1 ml) of sterile normal saline was inoculated aseptically into a Petri dish following the same procedure and incubated along with the inoculated plates.

Identification of fungal isolates from the Tigernut tubers

The fungal isolates were identified based on the macroscopic and microscopic characteristics by slide culture technique, and lactophenol staining, using the method of isolate identification based on the Watanabi (2010), methods. The fungal isolates were incubated at 37^0 C for about 72-120 hours. The morphology of the fungal isolates under the microscope was compared with the descriptions of Geo (Geo, *et al.*, 2003).

Biochemical Characterization:

Biochemical characterization of isolates involves the use of various biochemical tests to identify and classify microorganisms based on their metabolic and physiological characteristics. These tests provide valuable information about the biochemical reactions that occur within the cells of the microorganisms, aiding in their identification and differentiation. This process helps in determining the genus, species, or strain of the isolated microorganism. The results of these tests, combined with other factors like colony morphology and growth characteristics, help in the identification and classification of microorganisms.

Antibiotics Susceptibility Testing:

Antimicrobial susceptibility testing was performed using Mueller-Hinton (MH) agar (Oxoid, UK) plates by the Kirby-Bauer disk diffusion method according to the standard procedure from the Clinical Laboratory Standard Institute (CLSI) criteria (CLSI, 2022). The antibiotics tested in this study include; Ciprofloxacin, Levofloxacin, Gentamicin, Azithromycin, Ceftriaxone, and Meropenem to target Gram-negative pathogens while Penicillin, Amoxicillin, and Vancomycin, Clindamycin, Erythromycin, and Linezolid against Gram-positive pathogens. All antibiotic disks were procured from Oxoid, UK. Inoculated plates were incubated at 37°C, for about 18-24 hours, and zones of inhibition were measured in millimeters (mm) using a meter rule as stated in the CLSI criteria for Antibiotics susceptibility testing.

RESULTS

Biochemical characterization showed the presence of the following pathogens in the tiger nut purchased from these locations. *Bacillus subtilis*, *staphylococcus aureus*, *Aspergillus flavus*, *A. Niger, Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella, typhi*, *Enterococcus spp.*, and *Escherichia coli.*

Table 1: Biochemical Identification of Isolates

Isolates	G.R	Shape	LT	MTT	MT	VP	CT	OX	CG	UR	GL	CR	IND	Probable Organism
1	-	Rod	_	Nil	-	-	+	+		_	+		-	*Pseudomonas sp.*
2	+	Rod	–+	+	-	+	+	–+		-	+		-	*Bacillus spp.*
3	-	Rod	+	-	-	+	+	-		+	+	+	-	*Klebsiella pneumoniae*
4	+	Cocci	+	-		+	-				+	-		*Enterococcus spp*
5	-	Rod		+	+	-	+	-		-	+	-	-	*Salmonella, typhi,*
6	+	Cocci	-	-	-	+	+	-	+	+	+	+	-	*Staph. aureus,*
7	-	Rod	+	+	+	-	+	-		-	+		+	*Escherichia coli.*

The results of each biochemical test used in pathogen identification. Each procedure was carried out using the Standard guidelines. It would be helpful for you to become familiar with the list of test results and their corresponding abbreviations. These include (-) for negative, (+) for positive, G.R. for Gram reaction, LT for Lactose fermentation, MTT for Motility Testing, VP for Voges Proskauer, CT for Catalase testing, OX for Oxidase, CG for Coagulase, UR for Urease, GL for Glucose, IND for Indole, and CR for Citrate. It's important to take note of each abbreviation.

Table 2: Frequency of Occurrence of Isolates by Location

Isolates	**Ahia ofuu**	**PCG**	**Ishieke market**	**Ogbehausa**	**Intl market**	**Total**
Aspergillus flavus	5	3	3	2	5	18
Aspergillus niger	0	2	4	2	1	9
Pseudomonas sp.	7	4	1	8	2	22
Bacillus spp.	2	4	5	4	6	21
Klebsiella pneumoniae	2	1	4	3	5	15
Salmonella typhi,	3	4	5	4	3	19
Staph. aureus	8	5	7	4	6	30
Enterococcus spp	2	0	4	3	1	10
E. coli	5	7	6	11	8	37
Total	34	30	39	41	40	181

Table 2: showing the frequency of occurrence, and the sum of each Isolate per location, the total number of Isolates from each location of sample collection.
Key: PCG = Presco campus gate.

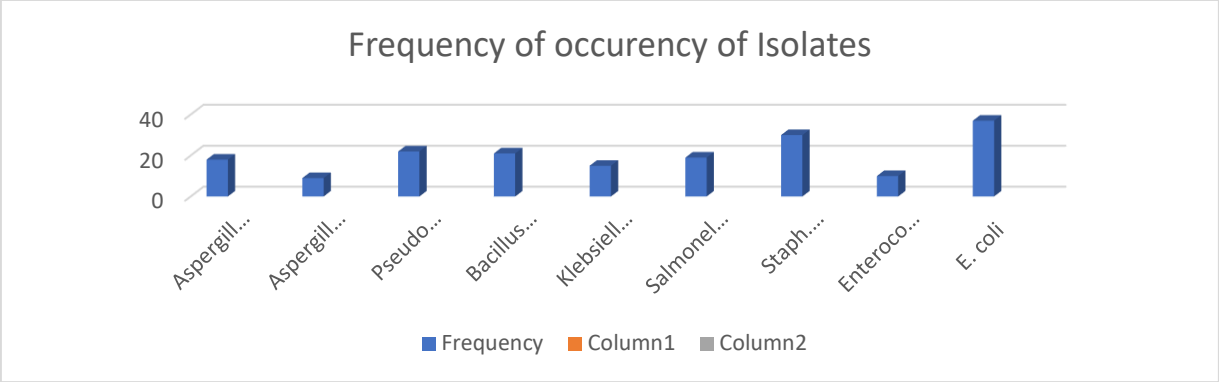

Figure 1: Chart representation of the total number of each isolate.

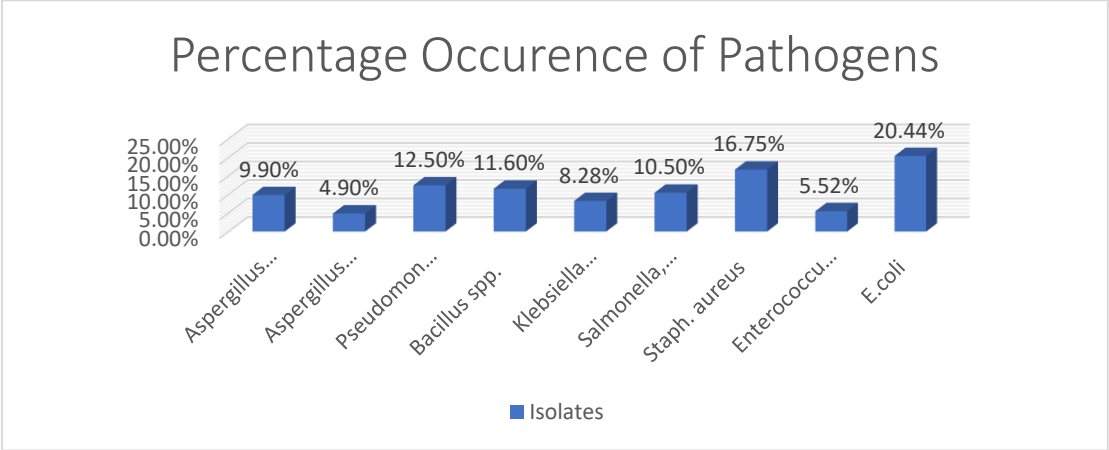

Figure 2: Chart representation of Isolates from the sites of sample collection.

Ogbehausa from the chart can be observed as having the highest number of pathogens from the sample collected. Figure 2 shows the percentage occurrence of each pathogen isolated from the tiger nut from the different locations. The isolate with the highest percentage of occurrence was *E.coli* (20.44%), *S. aureus* (16.75%), *Pseudomonas* (12.50%), *Bacillus spp*. (11.60%), *S. Typhi* (10.50%), *A. flavus* (9.90%), *K. pneumoniae* (8.28%), *Enterococcus* (5.52%), and *A. Niger* (4.90%) had the lowest percentage of occurrence.

Table 3: Antibiotics Susceptibility Pattern of the Bacterial Isolates (Percentage Antibiotics Susceptibility)

Isolates	CIP	LEV	GEN	AZT	CFX	MRP	CLN	ERY	PEN	AMX	VAN	LNZ
	(% Susceptibility)											
Pseudomonas sp.	50	64	68	55	59	54	23	32	22	18	27	27
Bacillus spp.	66	9	19	23	9	28	76	67	76	75	71	67
K. pneumoniae	47	60	53	66	46	60	13	47	27	20	26	33
Salmonella, typhi,	74	42	63	32	42	32	47	42	47	58	37	26
Staph. aureus	60	7	13	20	27	3	63	70	56	58	80	45
Enterococcus spp	54	27	13	18	13	22	68	72	59	81	63	59
E. coli	14	65	76	49	65	57	27	46	16	14	24	35

Keys: % = percentage of overall pathogen susceptible to each antibiotic tested.
CIP= Ciprofloxacin, LEV= Levofloxacin, GEN = Gentamicin, AZT= Azithromycin, CFX = Ceftriaxone, MRP= Meropenem, PEN = Penicillin, AMX = Amoxicillin, VAN = Vancomycin, CLN = Clindamycin, ERY = Erythromycin, LNZ = Linezolid

For Table 3**,** the table above shows the antibiotics tested against the isolates and the response of the isolates to each antibiotic. It can be observed that *Pseudomonas aeruginosa* had the highest susceptibility to Gentamicin at 68% and the highest resistance to Amoxicillin at 18%. *Bacillus spp*. showed the highest susceptibility to Clindamycin at 76%, while being most resistant to Levofloxacin and Ceftriaxone, both at 9%. *K. pneumoniae* exhibited the highest susceptibility to Azithromycin at 66%, but the highest resistance to Clindamycin at 13%. *S. typhi* showed high susceptibility to Ciprofloxacin at 74% and resistance to Linezolid at 26%. *S. aureus* displayed high resistance to Meropenem and Levofloxacin, with 3% and 7% susceptibility, respectively. However, it had 80% sensitivity to Vancomycin, and 70% to Erythromycin. *Enterococcus* and *E. coli* showed higher susceptibility to Amoxicillin, and Gentamicin, respectively, and resistance to Gentamicin and Ceftriaxone, both at 13% for *Enterococcus*, and 14% for Ciprofloxacin and Amoxicillin in the case of *E. coli.*

DISCUSSION

The study conducted on the microbial quality of tiger nuts sold in different locations in Abakaliki has revealed some concerning results. It was found that tiger nut milk sold in these locations contains several species of pathogenic microorganisms in the various samples purchased. Some of the pathogens identified include *Staphylococcus aureus, Escherichia coli, Salmonella spp, Bacillus subtilis, Klebsiella spp, Pseudomonas spp, Aspergillus flavus,* and *Aspergillus niger*. This finding is consistent with the results of Austin (2020) and Udeozor and Awonorin (2016), who also isolated fungal pathogens such as *Aspergillus spp.* It is important to note that further measures should be taken to ensure the safety of tiger nut milk for consumption. Figure 2, shows the percentage occurrence of each pathogen isolated from the tiger nut from the different locations. The isolate with the highest percentage of occurrence was *E.coli* (20.44%), *S. aureus* (16.75%), *Pseudomonas* (12.50%), *Bacillus spp*. (11.60%), *S. typhi* (10.50%), *A. flavus* (9.90%), *K. pneumoniae* (8.28%), *Enterococcus* (5.52%), and *A. Niger* (4.90%) had the lowest percentage of occurrence. The results obtained can be linked to the research conducted by (Nyarko, *et al.,* 2011). on the evaluation of microbiological safety in tiger nuts within the Cape Coast region of Ghana. Their findings revealed that the most commonly found species were *E. coli* and *Bacillus spp*., both of which accounted for 18.9%. Bacillus are bacteria that produce spores and are commonly present in soil, while *E. coli* is typically found in water (as a result of contamination from faecal matter in soil water) and on vegetables. The presence of these bacteria in food samples in this study may be inevitable because the spores of some strains of these organisms are resistant to pasteurization temperature. The study revealed that *Enterococcus spp*. accounted for 16.2% of the pathogens identified, while both *S. aureus* and *P. aeruginosa* were responsible for 13.5% each, and *Streptococcus spp*. for 10.8%. It is possible that *S. aureus*, a commonly found environmental bacterium, was introduced through cross-contamination during processing. It should be noted that *S. aureus* is known to produce an enterotoxin that plays a significant role in causing food-borne illnesses (Udeozor, and Awonorin, 2014). Table 3 provides information on the susceptibility patterns of various pathogens. Pseudomonas was found to be 68% susceptible to Gentamicin while being only 18% susceptible to Amoxicillin. *Bacillus spp.* showed the highest susceptibility to Clindamycin with a percentage of 76%, and the highest resistance to Levofloxacin and Ceftriaxone at 9%. *K. pneumoniae* had the highest susceptibility to Azithromycin at 66% and the highest resistance at 13% susceptibility to Clindamycin. *S. typhi* showed high susceptibility to ciprofloxacin at 74% and was resistant to Linezolid with a 26% susceptibility rate. *S. aureus* was highly resistant to Meropenem and Levofloxacin, with susceptibility rates of 3% and 7%, respectively. However, it showed 80% sensitivity to Vancomycin and 70% sensitivity to Erythromycin. *Enterococcus* and *E.coli* both showed higher susceptibility to Amoxicillin and Gentamicin, respectively. They were resistant to Gentamicin and Ceftriaxone at 13% each for *Enterococcus*, and 14% each

for Ciprofloxacin and Amoxicillin for *E.coli*. The presence and availability of high moisture content facilitate the proliferation of microbial populations, as substantiated by existing literature (Elmahmood and Doughari, 2011). The likely origins of these microorganisms within the samples can be attributed to the behaviour, and unhealthy practices of the vendors like sneezing, constantly washing their hands on the product in their usual practice of sprinkling water on the tiger nut tuber to prevent drying, along with the deposition of droplets resulting from coughing, talking, during the packaging and processing phase (Ojokoh and Tabowei, 2002). The presence of fungal pathogens *A. flavus, A. Niger* in tiger nut samples raises concerns due to its association with the spoilage, and contamination potential of the microbial community inhabiting storage environments. The spoilage process, predominantly characterized by the emergence of undesirable flavours, becomes evident in refrigerated tiger nut products. A comprehensive investigation carried out by Chukwu *et al.* (2013), demonstrated the presence of various fungal species, namely *Aspergillus niger, A. flavus*, and *A. terreus*, in both fresh and dried tiger nuts, and highlighted their potential resilience to processing treatments. Consequently, the increasing acidity over time may exacerbate the deterioration of the beverage, as bacterial growth and reproduction tend to thrive in acidic media.

The present study on the microbial quality of tiger nuts sold in various locations in Abakaliki has revealed the presence of different pathogenic microorganisms in the samples. Isolates include *Staphylococcus aureus, Escherichia coli, Salmonella spp., Bacillus subtilis, Klebsiella spp., Pseudomonas spp., Aspergillus flavus,* and *Aspergillus niger*. This finding is consistent with previous studies by Austin (2020) and Udeozor and Awonorin (2014), which also reported the presence of fungal pathogens such as *Aspergillus spp.* Figure 2 illustrates the percentage occurrence of each pathogen isolated from tiger nuts across different locations. The highest percentage of occurrence was observed for *E. coli* (20.44%), followed by *S. aureus* (16.75%), and *Pseudomonas spp*. (12.50%), *Bacillus spp*. (11.60%), S. Typhi (10.50%), *A. flavus* (9.90*%), K. pneumoniae* (8.28%), *Enterococcus* (5.52%), and *A. niger* (4.90%). These findings align with the research conducted by Nyarko *et al.* (2011) on the microbiological safety of tiger nuts in the Cape Coast metropolis of Ghana, where *E. coli* and *Bacillus spp*. were the most commonly encountered species. The presence of these bacteria in the study samples may be inevitable due to the resistance of some strains to pasteurization temperatures. Other pathogens identified in the study included *Enterococcus spp*. (16.2%), *S. aureus* and *P. aeruginosa* (13.5% each), and *Streptococcus spp*. (10.8%). *Staphylococcus aureus*, a common environmental bacterium, may have been introduced through cross-contamination during processing. This bacterium is known to produce enterotoxins that can cause foodborne illnesses Udeozor and Awonorin, 2014). Table 3 displays the susceptibility pattern of the pathogens. *Pseudomonas spp.* exhibited the highest susceptibility to Gentamicin (68%) and the highest resistance to Amoxicillin (18%). *Bacillus spp.* showed the highest susceptibility to Clindamycin (76%), while being most resistant to Levofloxacin and Ceftriaxone (9% each). *K. pneumoniae* was most susceptible to Azithromycin (66%) and showed the highest resistance to Clindamycin (13%). *S. typhi* displayed high susceptibility to Ciprofloxacin (74%) but was resistant to Linezolid (26%). *S. aureus* demonstrated high resistance to Meropenem and Levofloxacin, with susceptibility rates of 3% and 7% respectively, while showing 80% sensitivity to Vancomycin and 70% to Erythromycin. *Enterococcus* and *E. coli* exhibited higher susceptibility to Amoxicillin and Gentamicin, respectively, while showing resistance to Gentamicin and Ceftriaxone (13% each for *Enterococcus*) and resistance to Ciprofloxacin and Amoxicillin (14% each for *E. coli*). The presence and availability of high moisture content facilitate the proliferation of microbial populations, as supported by existing literature (Elmahmood and Doughari, 2007). The likely sources of these microorganisms within the samples can be attributed to the soil, water, and the vendors' behaviours and unhealthy practices, such as sneezing and constantly washing their hands over the product as a preventive measure against drying. Additionally, droplets resulting from coughing and talking during the packaging and processing phases may contribute to contamination (Ojokoh and Tabowei, 2002). The presence of fungal pathogens like *A. flavus* and *A. niger* in tiger nut samples raises concerns due to their association with spoilage

and the potential contamination of storage environments. The spoilage process, characterized by the emergence of undesirable flavours, becomes evident in refrigerated tiger nut products. A comprehensive investigation conducted by Chukwu *et al.*, (2013), (Akomolafe and Awe (2017), demonstrated the presence of various fungal species, including *Aspergillus niger, A. flavus*, and *A. terreus*, in both fresh and dried tiger nuts, highlighting their resilience to processing treatments. Chidiebere *et al. (*2017) suggested that these microbial loads can be reduced by washing food products with running water. Consequently, the increasing acidity over time may worsen the deterioration of the beverage, as bacterial growth and reproduction tend to thrive in acidic environments.

CONCLUSION

The presence of pathogenic microorganisms in tiger nuts sold in different locations of Abakaliki emphasizes the need for proper hygiene practices during the cultivation, processing, and handling of these products. Additionally, awareness programs and strict adherence to food safety regulations are crucial to prevent the spread of foodborne illnesses associated with contaminated tiger nut consumption. This study provides valuable insights into the microbiological quality of tiger nuts in Abakaliki, highlighting the importance of ongoing monitoring and quality control measures to ensure the safety of this popular food commodity.

RECOMMENDATIONS

To mitigate the potential risks associated with microbial contamination in tiger nuts sold around the campus area, several recommendations can be implemented. Strict hygiene protocols should be implemented during the processing of tiger nut tuber. Measures such as thorough cleaning and sanitization of equipment, and proper hand hygiene for vendors. Adequate refrigeration and temperature control measures should be implemented to prevent microbial growth and reduce the risk of contamination. Vendors should establish dedicated areas and utensils for the preparation of tiger nut milk, minimizing the chances of cross-contamination with other food products. Tiger nut vendors should endeavour to avoid groundwater sources while sourcing water for washing tiger nut tubers.

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