MICROBIAL AND PHYSICOCHEMICAL ASSESSMENT OF ROAD-SIDE ROASTED PLANTAIN SOLD IN ISOKO NORTH LOCAL GOVERNMENT AREA,Nigeria

ABSTRACT

A research to investigate microbes present in roadside roasted plantain sold openly and freely in four different locations in Isoko North local government area, Delta State was analyzed. Samples were analyzed for bacteria and fungi using standard microbiological procedures. Five bacteria species and three fungi species were isolated from the samples; *Corynebacterium species, Proteus mirabilis, Staphylococcus aureus, Vibrio species, Bacillus subtilis, Candida albicans, Mold species and Rhizopus oryzae* respectively. The total bacteria heterotrophic plate count ranges from 7.6 $\times 10^3$cfu/g to 47.6 $\times 10^4$cfu/g. *Proteus mirabilis* has the highest heterotrophic plate count while *Vibrio species* has the least count. *Proteus mirabilis* has the highest percentage of occurrence of 43.50% while *Corynebacterium species, Staphylococcus aureus* and *Bacillus subtilis* has the least percentage occurrence of 25%. Statistical test reveals no significant difference ($P>0.05$) between bacteria count in heated samples A and cold samples B. *Candida albicans* and *Mold species* were the highest fungal isolate with the percentage occurrence of 43.75% while *Rhizopus oryzae* has the least percentage occurrence of 12.5%. The total heterotrophic plate count for fungi isolates ranges from 2.5 $\times$ 10^3cfu/gto 12.6 $\times$ 10^4cfu/g. Statistical test reveals no significant difference ($P>0.05$) between fungi count in heated samples A and cold samples B due to heat resistance of these microorganisms. The physicochemical parameters analyzed were potassium, sodium, chloride, nitrate, and nitrite. Nitrite has a leading value than other chemical value. This can be due to post production or heat applied to samples. This study shows that road side roasted plantain contains a variety of microbes which may be dangerous or may have some health implications on consumers especially *Proteus mirabilis* which was obtained from this study.

Key words: Microbial, Physicochemical, Assessment, Road-side, Roasted, Plantain

INTRODUCTION

“Roasted plantains are ready to eat foods prepared mainly and sold by vendors and hawkers in the street and other similar public places” (FAO, 1997). “Extensive sales of roadside roasted plantains as food, as in most other areas, arises from multiple causes migration to the cities, long commuting distances between the workplace and home, lack of cooking knowledge, or absence of establishment that serve priced food close to workplace” (Tinker, 1997; Maxwell, 2000). “Roasted plantain is one of the ready to eat food for immediate consumption at the points of sale” (Adesiyun, 1995). “Roasted plantain provides a major source of income for a vast number of persons, particularly women, a chance for self-employment and the opportunity to develop business skills with low capital investment, least expensive and must accessible means of obtaining a nutritionally balanced meal outside the home for money low income people” (WHO, 2002; Dipeolu*et al.,* 2007).

“Roadside roasted plantains we purchase are not sterile in the sense that they normally contain germs (bacteria, fungi, etc.), some of which can lead to intoxication and infections when present above the acceptable level” (Agbodaze*et al.,* 2005). “Roasted plantain harbors a variety of micro-organisms, bacteria and fungi which are ubiquitous and are especially plentiful in soil and around us (air) and could easily contaminate foods”. (Ashenafi, 1995). “This could also result from improper protection of food (roasted plantain) from flies, which many carry foods borne pathogens or diseases such as diarrhea” (Tambekar*et al.,* 2008). “Contamination and growth of pathogens such as *Staphylococcus aureus*, *Salmonella species, Bacillus species, Pseudomonas aeruginosa, Clostridium species, Vibrio cholerae* and *Escherichia coli* can result in perceptible changes in quality of the plantain” (Rath and Patra, 2012; Arijit *et al.,* 2010 and (Oranusi and Braide, 2012). “Some of the road side roasted plantains can transmit a wide range of disease in a condition termed food infection, where the food serves as a vehicle for the transfer of the pathogen to the consumer in whom the pathogen grow and cause disease” (Avashia, 2007).

Food borne illness caused by microbial contamination of roasted plantain is an important international public health problem with consequent economic reduction (Bryan *et al.*,1992) and is known to be a major cause of diarrhea especially in developing countries (Ossai, 2012; Falola, *et al.,* 2011). “However, in Nigeria, several foods such as the roadside roasted plantain have been reported to have high incidence of bacteria” (Mead, 1994).

This present study examines and isolate micro-organisms from roadside roasted plantain obtained from different vendors and access the physicochemical properties of roasted plantain in Isoko north L.G.A. Delta State.

MATERIALS AND METHOD

Study Area

This research was conducted in Isoko North Local Government Area, Delta State of Nigeria, the people are Isoko speaking and hospitable. Their main activities are farming accompanied by some hunting and trading. The areas covered are becoming densely populated and fast growing as well high social activities being boosted by a tertiary institution (Delta State Polytechnic, Ozoro). The region experiences higher rainfall and humidity most of the year.

Study size

The samples that was used for this research were collected from different vending points in Isoko North Local Government Area.

Samples Collection

A total of Thirty-two (32) samples comprising of two different kind of roasted plantain, grouped into sample A and B were accessed microbiologically. These samples were collected from different areas in Isoko North LGA (Ozoro, Emevor, Owhelogbo, and Ofagbe) in the same quantity. The samples were purchased in a sterile polythene bag (nylon) enveloped directly from vendors and immediately it was moved to the laboratory for analysis. Some samples that were displayed immediately after roasting and cool off while the others will be obtained directly from the fire. All together thirty (32) plantain samples of two different kind of hot and cold sold in the study area will be examined.

Table 1: Distribution of Plantain Samples in the Study

ZONES	PLANTAIN SAMPLES		TOTAL
	SAMPLE A	**SAMPLE B**	
Ozoro	4	4	8
Emevor	4	4	8
Owhelogbo	4	4	8
Ofagbe	4	4	8
TOTAL	**16**	**16**	**32**

KEY: sample A: hot, sample B: cold

Sterilization Glass ware:

The glass wares that were used for this experiment was washed with detergent rinsed thoroughly and sterilized using autoclave at 121^0C for 15mins

Methods

Culture of Samples

Procedures

10g of the plantain samples A and samples B were weighed into a sterile mortal and was thoroughly pounded, thereafter it was placed in a sterile test tubes which was labeled on each of the petri dish bearing the locations it was obtained from. Nutrient agar, MacConkey agar, Pseudomonas agar were used to isolate bacteria species while Potato dextrose agar (PDA) will be used for fungi. Weighing balances will be used to measure the weight of the agar and afterward 100ml of distilled water was added to each agar in a conical flask (100ml of distilled water will be added to 2.8g of nutrient agar; 100ml of distilled water will be added to 3.8g of potato dextrose Agar (PDA) using the method of Cheesbrough (2006). Media prepared will be according to the manufacturer instruction and then it will be used for enumeration of isolated bacteria.

The plates were incubated at 37^0C for 24 hours for bacterial and at 23^0C for 3-7 days for fungi. Growth of organisms were identified according to their morphological characteristics and reactions to biochemical test for bacteria and cultural morphology for fungi

Sub-Culturing of the Culture

The colonies of the bacteria obtained in the culture was sub cultured into a fresh nutrient agar.

Identification of organisms isolated

Organisms isolated from the culture media after overnight incubation; were identified using the following methods

a. Gram staining techniques
b. Biochemical test

Gram Staining Techniques

"The gram staining reaction was used to help identify isolates in culture (gram positive or gram-negative organisms) using a sterile wire loop, a pure culture from a discrete colony on the culture plate was emulsified in a drop of normal saline on grease free clean microscope slides" [23].

Procedures

- The dried smear was fixed
- The fixed smear was covered with crystal violet stain for 60 sec
- The stain was rapidly washed off with clean water
- All the water was tipped off, and the smear covered with lugol's iodine for 60 sec (1 min)
- The iodine was washed off with clean water.
- The smear was decolorized rapidly for few seconds using 95% alcohol for 15secs and washed immediately with clean water.
- The smear was covered with neutral red stain for 2mins
- The stain was washed off with clean water.
- The back of the slide was washed clean and placed in a draining rack for the smear to air dry
- The smear was then examined microscopically with oil immersion and X100 objective to report the bacteria and cells

Result:

Gram positive bacteria - Dark Purple

Gram negative bacteria - Dark Red

Motility test

Knowing whether an organism is motile or non-motile can often assist in its identification; here the hanging drop method was used from broth culture of the isolates.

Hanging drop preparation:

1. A drop suspension was placed on a cover glass and inverted over a normal slide supported on ring plasticine.
2. The preparation was microscopically examined for motile organisms using 10x and 40x respectively.

Note: True bacteria motility is the ability of an organism to move itself in different directions or a single direction.

Biochemical Test

a. Triple sugar iron Agar test
b. Indole test
c. Glucose test
d. Citrate test
e. Lactose test
f. Catalase test
g. H_2S
h. gas

The biochemical analyses were in accordance with procedures reported by Cheesbrough (2006).

a) **Triple sugar iron Agar test (TSI):**

Bacteria isolate was stabbed into TSI slant media and also streaked on the surface of the slant after which the medium was incubated at optimal temperature of 37^0c for 24 hours.

b) **Citrate test:**

This test is used occasionally to assist in the identification of enterobacteria. It is based on the ability of an organism to use citrate as its only source of carbon and ammonia as its only source of nitrogen.

Procedures:

The bacterial isolates were inoculated from a Simmons citrate of the test organism and incubated for 24 hours at 37^0

Result:

The blue color and streak of growth indicated a positive reaction while the original green color and no growth are indicative of a negative reaction (Bello, 2002).

c) **Indole test:**

This test demonstrates the ability of certain bacteria to decompose the amino acid tryptophan to indole which then accumulates in the medium for indole production

Procedures

Bacterial isolates were inoculated into peptone water medium contained in sterile test tubes inoculated at 37^0c for 48 hours after the incubated at 37^0c for 48 hours. After the incubation period about 3 drops of kovac's indole reagent was added to the peptone water culture. The bottles were shaken thoroughly and allowed to stand and observed for color development.

Result:

A Red Color ring at the interface of the medium denotes a positive result. And if the isolate is negative, the reagent layers will remain yellow or slightly cloudy (Bello, 2002).

d) **Catalase test:**

This test detects the presence of catalase enzymes when present in bacterium, it catalyzes the breaking down of hydrogen peroxide with the release of oxygen as bubble.

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

Procedures:

With a wire loop, a colon was picked from the pure culture and was transferred to the center of a glass slide 1 – 2 drops of 3% peroxide was added to the bacterial isolates.

Result:

Immediate production of bubbles indicated positive result and if no bubble indicated negative.

a. Glucose test: if the bottom of the media turn from pink to yellow (glucose positive)
b. Lactose test- if the top of the media turn from pink to yellow (lactose positive)
c. Gas test- if bubble is present in media (gas positive)
d. H_2S - if black is present in the media (H_2S positive)

Note: T.S.I slant medium was used to check for the present of the above test

PHYSICOCHEMICAL DETERMINATION

a. Determination of chloride content
b. Determination of potassium content
c. Determination of sodium content
d. Determination of nitrate content.
e. Determination of nitrite content.

Procedure:

The chloride, potassium, sodium, nitrite and nitrate content were carried out using spectrophotometer method, the procedure involves preparing a blank (deionized water) for the zeroing of the spectrophotometer. A standard was prepared (a known concentration of chloride, potassium, sodium, nitrite and nitrate) while the samples to analyze are the unknown. The blank, standard and sample was poured into three different corvettes each. The blank will be placed inside the first corvette compartment, follow to standard in the next compartment and the samples was placed on the third corvette compartment. The chloride content was displayed in digital form and was recorded. The same procedure will be repeated for potassium, sodium, nitrite, and nitrate respectively.

Data Analysis

Results obtain will be analyzed using SPSS software, version 20 (IBM, USA) for both the descriptive and inferential analysis. Results will be expressed as mean and standard deviation.

One-way analysis of variance (ANOVA) will be used to determine the level of significance between the parameters. Level of significance will be set at $P \leq 0.05$.

RESULT AND DISCSSION

Table 2: Shows Cultural, Morphological and Biochemical Characteristic of Bacteria Isolates.

Isolates	Gram stain	Morphological characteristic	Citrate	Catalase	In dole	Glucose	lactose	H^2S	Gas
Bacillus subtilis	GPB	RODS	-	+	-	+	+	-	-
Corynebacterium species	GPB	Rods	-	+	-	+	-	-	+
Proteus mirabilis	GBN	Rods	+	+	+	+	-	-	+
Staphylococcus aureus	GPC	Cocci	-	+	+	+	+	-	+
Vibrio species	GNB	Rods	+	+	-	+	-	-	+

Key = + = positive, - = negative, GPB = Gram positive Bacillus, GNB = Gram

Negative bacillus, GPC = Gram Positive Cocci

Table 3: Shows bacterial isolates, number of occurrence and percentage of occurrence

SAMPLE	Bacterial Isolates	Numbers of	Percentage (%) of

		Occurrence	Occurrence
A	*Corynebacterium species*	2	25.00
	Proteus mirabilis	3	37.50
	Staphylococcus aureus	2	25.00
	Vibrio species	1	12.50
B	*Bacillus subtilis*	2	25.00
	Proteus mirabilis	4	50.00
	Vibrio species	2	25.00

Table 4: Bacterial Isolates and its Total Heterotrophic Plates count from the Samples

Sample	Total heterotrophic plate count (cfu/g)
A1	15.6×10^4
A2	28.4×10^4
A3	32.0×10^4
A4	20.6×10^4
A5	15.6×10^4
A6	22.1×10^4
A7	7.6×10^3
A8	13.5×10^4
B1	31.3×10^4
B2	21.2×10^4
B3	47.6×10^4
B4	29.7×10^4
B5	42.1×10^4
B6	16.4×10^4
B7	26.8×10^4
B8	18.5×10^4

Table 5: Morphological and Microscopic Character of the Fungal Isolates

Morphological characteristic	Microscopic characteristics	Micro- organism
Growth rate is very rapid, and colonies are typically cotton- candy like in texture the surface colony color is initially white becoming gray to yellowish brown in time while reverse is white to pale	Non- septate or scarcely septate broad hyphae with diameter ranging from 6-15Nm,rhizoids,sporangiophores, sporangia and sporangiospores are present sporangiospores are present,sporangiophores usually unbranched, brown in color, solitary or appear in clusters	*Rhizopus Oryzae*
The colony was creamy without profuse growth	Hyphae and conidiospore are non-septate	*Candida Albicans*
Colony is typically cotton candy like in texture. The surface colony color is initially white them becoming gray	Hyphae a fuzzy appearance and transparent, mycillium appear like very fine, flutty white over the surface	*Mold Species*

Table6: Shows Fungi Isolates, number of occurrence and percentage of occurrence.

Sample	Bacterial Isolates	Number of occurrences	Percentage (%) of occurrence
A	*Candida Albicans*	5	62.50
	Mold Species	3	37.50
B	*Candida Albicans*	2	25.00
	Mold Species	4	50.00
	Rhizopus Oryzae	2	25.00

Table 7: Shows the occurrence of Fungi Isolates and its total Heterotrophic count.

Samples	Total Heterotrophic Count
A1	12.6×10^{4}
A2	3.9×10^{3}
A3	11.2×10^{4}
A4	6.8×10^{4}
A5	5.9×10^{4}
A6	9.3×10^{4}
A7	11.9×10^{4}
A8	4.9×10^{4}
B1	11.8×10^{4}
B2	10.8×10^{4}
B3	2.5×10^{3}
B4	11.5×10^{4}
B5	9.7×10^{4}
B6	5.9×10^{4}
B7	10.7×10^{4}
B8	7.6×10^{4}

TABLE 8:Physicohemical Analysis Result

	SAMPLE A								SAMPLE B							
PARAMETERS	1A	A2	A3	A4	A5	A6	A7	A8	B1	B2	B3	B4	B5	B6	B7	B8
Potassium	0.782	0.780	0.74	0.778	0.760	0.770	0.790	0.796	0.633	0.857	0.645	0.786	0.838	0.679	0.840	0.798
Sodium	1.031	1.059	1.040	1.039	1.056	1.038	1.042	1.048	0.608	0.875	0.786	0.679	0.870	0.789	0.842	0.865
Chloride	1.439	1.457	1.402	1.440	1.437	1..430.	1.450	1.456	0.976	0.661	0.960	0.908	0.736	0.620	0.687	0.920
Nitrite	1.530	1.642	1.528	1.523	1.640	1.529	1.649	1.629	0.533	1.258	0.529	0.520	1.239	1.220	0.530	0.525
Nitrate	0.670	0.763	0.750	0.761	0.763	0.759	0.761	0.760	0.510	1. 030	0.520	0.590	1. 020	1.018	1.029	0.509

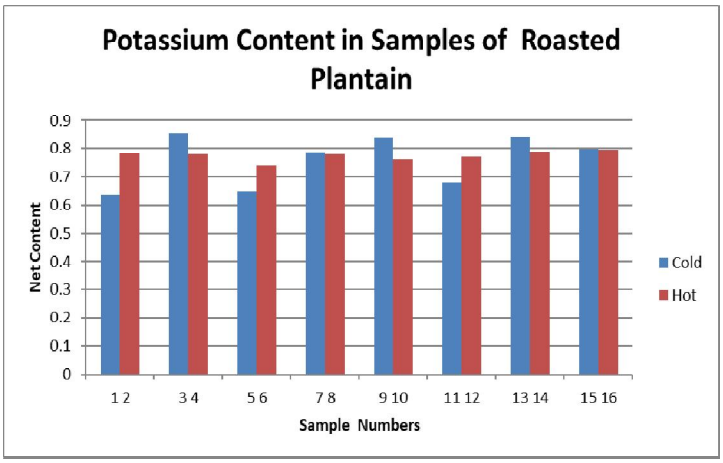

Fig 1: Potassium content in roasted plantain

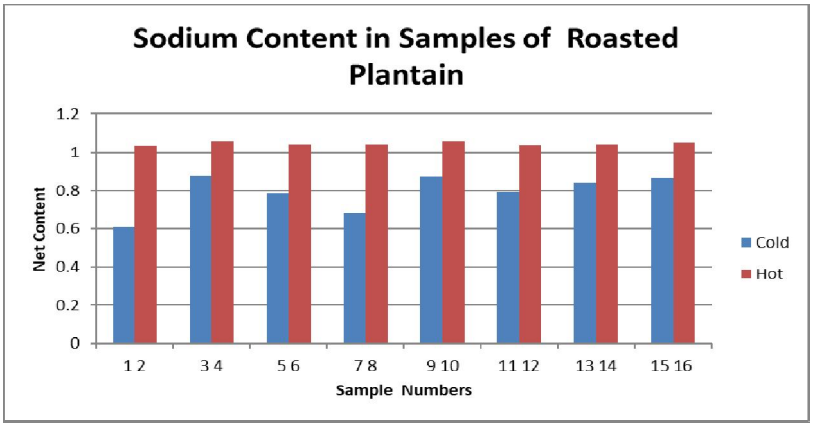

Fig 2: Sodium content in roasted plantain

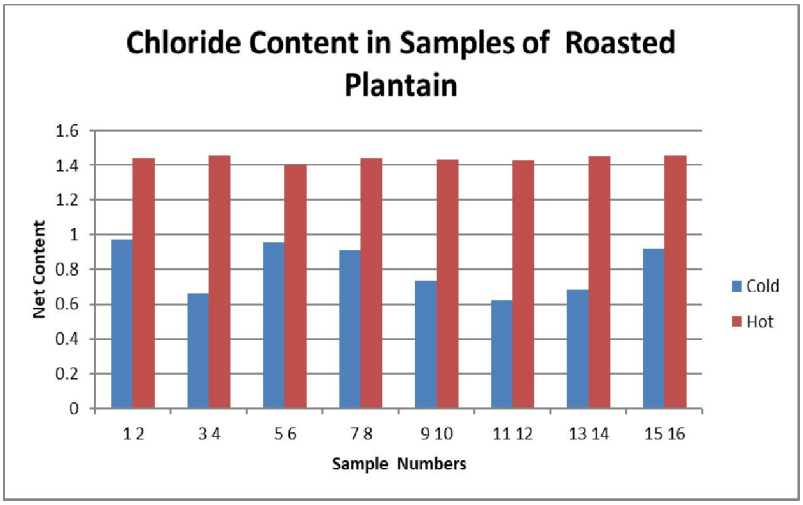

Fig 3: Chloride content in roasted plantain

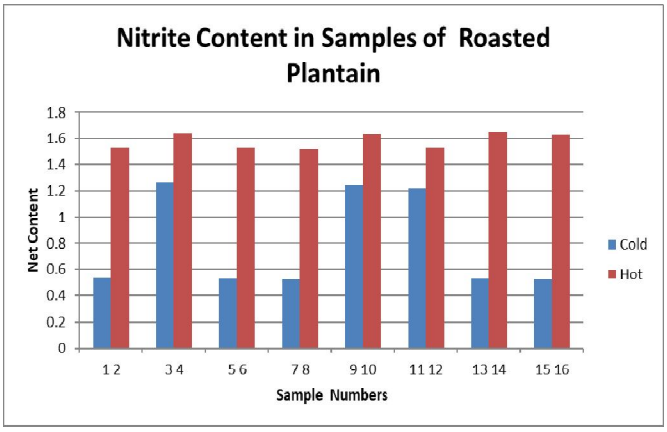

Fig 4: Nitrite content in roasted plantain

This study revealed that the roadside roasted plantain is contaminated with different bacteria and fungi species and as well a high chemical composition. Table 2 shows the cultural morphological and biochemical characteristics of bacteria isolates and their ability to utilize sugar as the substrate. The bacterial isolated from sample A and sample B where *Bacillus subtilis, Corynebcateriun species, Staphylococcus aureus, Proteus mirabilis* and *Vibrio species*. These were the most common bacteria isolates identified in roadside roasted plantain.

The presence of *Staphylococcus aureus* in the roadside roasted plantain is indicative of human activities contamination after production. This could be from direct handling of the food which may result from the microorganism on the surface of the skin, mouth, nose, coughing and sneezing from the vendors (Okonko, *et al.,* 2008; Sobuko*et al.,* 2009).

Table 3 shows that all the bacteria isolated occurred at a given numbers and at a given percentage both on sample A and sample B. *Proteus mirabilis* has the highest contamination rate in both sample A and B; with the percentage rate of 87.50% and numbers of occurrence Seven (7) followed by *Vibrio species* with the percentage rate of 37.50% and numbers of occurrence three (3). The bacterial isolates with the lowest contamination rates in both sample A and sample B with the percentages of 25% and numbers of occurrence of 2 are *Corynebaterium species, Staphylococcus aureus and Bacillus subtillis.*

The presence of *Bacillus subtillis* is associated with the production of toxin, diarrhea and emetic in food which causes food poisoning. It is found mostly in roadside roasted plantain and can survive normal roasting as a heat resistant spore. (Rajkowski and Bennelt, 2003).

Table 4 shows the Bacteria isolates and their total heterotrophic plate counts. *Proteus mirabilis* has the highest heterotrophic plate counts both in sample A and in sample B with total coliform counts (cfu/g) of 32.0×10^4cfu/g in sample A and 47.6×10^4cfu/g in sample B. these bacteria could be of increase in sample B due to the fact that the sample was a cold type as seen from table 3 above. *Vibrio species* has the lowest heterotrophic plate in sample A with the total cfu/g count of 7.6×10^3cfu/g and has the lowest heterotrophic plate count of 16.4×10^4cfu/g in sample B. The presence of *Proteus mirabilis* contamination in roadside roasted plantain is association with production of pathogenic disease which has the highest risk of infecting consumers (food microbiology, 2009)

The result obtained in this study from bacterial viable count in terms of sample A and sample B shows that sample B has the highest contamination rate with the mean cfu/g count of 106.50×10^5cfu/g and with total heterotrophic plates count of 6.8×10^3 to 15.6×10^4 while sample A has the lowest contamination rate with the mean cfu/g count of 88.5×10^4cfu/g and total heterotrophic plate count of 7.6×10^3 to12.4×10^4cfu/g from table 4 and table 5 respectively. Statistical test reveals no significant difference ($P>0.05$) between bacteria count in heated sample A and cold sample B reason being that most microorganism are heat resistant and thrive under high temperature as seen by Wiess (1955) with *cornyebacterium species* surviving temperature of 62^OC for a 30minutes period and *Vibro species* surviving temperature up to 54^OC for a 5min period as reported by Shultzs*et al.*, (1983). This indicates that bacteria survival rate remains unchanged after contamination of samples as much roasted plantains are prepared under a cook time of 30minutes and growth as they are removed from heat and set within a warm temperature as revealed in this study.

The result obtained in table 6 shows the morphological and microscopic characteristics of fungi which gives rise to the different fungal isolated were *Candida albican, Mold species* and *Rhizopus oryzae* these are most common fungi isolates, the number at which they occurred and the percentage they occurred *Candida albican*has the highest numbers of occurrence and percentage rate which are 7 and sample B; likewise mold species. *Rhizopus oryzae* has which is 2 and 25.0% in sample B only.

The presence of *Rhizopus oryzae* in roadside roasted plantain is not surprising as they disperse in the form of spore and clusters which is abundant in the environment and can be introduce

through dust and soil (Apinis, 2003) their presence in this food is of serious public health concern as these fungi have all been implicated with the production of *Mycotoxin* (Makum*et al.*, 2009).

Table 7 shows the number of occurrence of fungi isolates and their total heterotrophic count. *Candida albican*have the highest heterotrophic count of 24.4×10^4 cfu/g both in sample A and sample b followed by mold species with the heterotrophic plate count of 14.7×10^4cfu/ml. *Rhizopus oryzae* has the lowest heterotrophic plate count of 2.5×10^3cfu/ml only on sample B. fungi grow best in warm temperature and some may grow better in very high temperature. Statistical test reveals no significant difference ($P>0.05$) between fungi count in heated sample A and cold sample B with cold sample generating higher counts because they are kept within favorable temperature conditions which is a better growth conditions for these microorganisms. This indicates that fungi growth in food after contamination remains unchanged and would multiply upon warm temperatures of 70-90^{O}F.

The highest incident of fungi contamination in roadside roasted plantain is not expected. This could be due to the excessive heating of food sample before purchase

Figure 1-4 shows the results obtained from chemical analysis of sample A and sample B. the chemicals analyzed were potassium, sodium chloride, nitrate and nitrite. Nitrite has a leading value than the other chemicals analyzed. Statistically analyzed samples reveals no significant difference in potassium and nitrate contents ($p>0.05$) and indicates that these contents are unchanged by the overall preparation process. Although significant difference ($p<0.05$) was seen for sodium, nitrate and chloride contents and these could be as a result of the preparation process which causes salt used in coating samples to leach and adhere samples and may be removed during serving processes as they are strapped off together with visible ash.

The result obtained in this study from chemicals determination in roadside roasted plantain is not expected. This could be due to the excessive heating of food sample

Microbial guideline for roadside roasted plantain stipulated is that “the plate count must be 10^7cfu/g, 1.0×10^4cfu/g from plantain products and for coliforms, the plate count must be 10 cfu/g” (Gubert, *et al.*, 2000). The microbial load of road side roasted plantain was higher than stipulated, hence their presence constitute a health risk, it can be adjudged that the roadside

roasted plantain retailed in Isoko North L.G.A. as obtained in this study are not bacteriologically fit for consumption due to unhygienic production practices and prolong exposure to environment.

CONCLUSION AND RECOMMENDATION

The result of the study indicates that most food pathogens are of soil or intestinal origin and are transferred through poor food preparation. Furthermore, the result obtained from this study show that road side roasted plantain contains a variety of microbes which may be dangerous or may have some health implications on consumers especially *Proteus mirabilis* which was obtained from this study. The excessive intake of roadside roasted plantain above the normal or expected level may lead to complication in the body chemistry such as developing gastro- intestinal.

From the above conclusion, it is recommended that; Basic training in food hygiene is recommended to ensure that food vendors follow the required rules for proper hygiene and sanitation. Training on hygiene and sanitation of food handlers on regular basic for carriers, the establishment of code of practice for the street food industry and provision of basic water and waste management utilities are recommended to diminish the gap between knowledge and practices of roasted plantain.

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