Original Article
ISOLATION OF MICROORGANISMS FROM READY TO EAT FOODS COLLECTED FROM TWO SELECTED RESTAURANTS IN TANKE OKE-ODO, ILORIN

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ABSTRACT

The microbial assessment of four ready to eat food samples collected from two restaurants in Tanke-Oke-Odo area, Ilorin was carried out. The physicochemical parameters such as pH, moisture content, titratable acidity and ash content were determined. Five bacteria and three species of fungi were isolated and identified. The bacterial isolates are *Staphylococcus aureus., Bacillus cereus, Escherichia coli, Salmonella* sp., and *Shigella* sp. The fungal isolates are *Aspergillus flavus, Mucor mucedo* and *Saccharomyces cerevisiae*. The beans sample collected from the second restaurant had the highest mean bacterial count of 2 x 10^4 cfu/g while the rice and beans sample collected from the first restaurant had the least mean bacterial count of 8.5 x 10^3 cfu/g. The amala sample collected from the second restaurant had the highest mean fungal count of 8.5 x 10^3 cfu/g while the beans sample collected from the first restaurant had the least fungal count of 2.0 x 10^3 cfu/g. The significance of the counts, the presence of microorganisms in the food and the improvement in sanitary practices during the preparation of the food samples are stressed.

Keywords: Microbial assessment, Physicochemical parameter and Bacteria count.

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INTRODUCTION

The term ready to eat food means food that is ordinarily consumed in the same state as that in which it is sold or distributed and does not include nut in the shell and whole, raw fruits and vegetable that are intended for hulling or washing by the consumer. These may include cooked rice, cooked beans, pounded yam, porridge, Eba, Amala, etc (ICMSF, 1986).

Food is usually composed of carbohydrates, fats, proteins and water that can be eaten or drunk by animal or human for nutrition or pleasure(Davidson, 2006).Foods are complex organic substances which living organisms require for producing energy for metabolic processes and for building up of body tissues when they are broken down within the body of a living organism they may be used for repair of worn out tissues and cells, replacement of dead old

cells, fight against foreign invaders(antigens), and proper functioning of body systems amidst a whole lot of other functions. Foods are important for metabolic processes in living organisms that lack of it or its inadequacy will result in diverse disorderliness and malfunctioning. Excess food can be detrimental to a living organism so likewise do contaminated foods have effects in living organisms when ingested (Alcamo, 1994). From the microbiological perspective, food can be viewed as a fertile ecosystem in which these organisms vie for their nutrients (Nester *et al.,* 2004).

The consumption of food is essential for human survival, but along with gaining good nutrition and satisfaction from eating food, occasionally human beings consume undesirable biological agents and toxins (Ali *et al.,* 2004). Food microorganisms and human beings have had a long and interesting association that developed long before the beginning of recorded history. Food is not only of nutritional value to those who consumed them but also often are ideal culture media for the growth of microorganisms (Willey *et al.,* 2008). Foods are normally contaminated with bacteria and others microbes since the environment in which we live is colonized by them.

The current concern in public health is food safety. Consumers are much aware of the potential for the large scale food borne outbreaks because of mishandling or improper processing of foods, and they are demanding a safer supply (WHO, 2000; WHO, 2011).

Foods prepared locally for human consumption is at greater risk of microbial contamination. The risk is highly variable in different people at different geographical locations. The hygiene procedures practiced during the preparation of food go a long way in reducing the risk of contamination and vice versa. Also, the health status of the individual preparing the food is a major determinant (Alcamo, 1994). A very good example of this is "typhoid Mary"-Mary Mallon, who was the most famous carrier of the typhoid bacteria. From the late 1980's till early 1990's, Mary Mallon worked as a cook and hence she was continually spreading the disease (Willey *et al.*, 2008).

In developing countries such as Nigeria, there are serious concerns about sanitation of ready to eat foods, particularly as potable water is seldom available at preparation venues and fast food stands and also most food handlers lack basic knowledge of proper personal and environmental hygiene (Bukar *et al.,* 2010).

Eating at local restaurants which could be canteen, cafeteria or restaurants is a common practice by students, staff and the general public. These local restaurants are located at different areas, some are very close to the road side, and some are located by dumping sites and bushes.

The aim and objective of this work was to study the microbiological quality of some ready to eat food mainly the ones mostly eaten such as rice, beans, amala and eba sold in two local restaurants of highly student populated area of Tanke, Oke-Odo area, Ilorin and how its quality is being related to the well being and health status of the consumers.

MATERIALS AND METHOD

Sterilization of materials

All materials used were adequately and appropriately sterilized before and after use. Glass wares such as test tubes, conical flasks, pipettes, etc were thoroughly washed with detergents, rinsed properly with water and drained. They were wrapped in aluminum foil and sterilized in hot air oven at 170^0C for 1 hour. Prepared media and distilled water were autoclaved at 121^0C for 15 minutes. Metal equipments like the inoculating loop were heated to redness in an open flame before and after use. The laboratory bench was always swabbed using 70% ethanol for disinfection before analysis was made. Every isolation and inoculation was done near the flame to reduce contamination of the agar plates tubes.

Collection of samples

Samples of four ready to eat food which are rice, beans, amala and eba were collected from two local restaurants in Tanke Oke-Odo area in Ilorin. The food samples were collected aseptically to avoid contamination, labeled appropriately and were immediately transported to the laboratory for analysis.

Physicochemical analysis

pH Determination

The pH of ach sample was determined in the laboratory using pH meter with glass electrode. Five grams of each sample was weighed and macerated with porcelain pestle and mortar. The macerated samples were dispersed in separate beakers which contains 50 milliliters of sterile distilled water. The suspensions were thoroughly mixed and left to settle

for ten minutes. After about 10 minutes, the electrode of the pH meter was dipped into the solutions one after the other without allowing it to touch the settled particles beneath. The pH value was recorded.

Moisture content determination

Five grams of each of the macerated sample was weighed with aluminum foil, labeled and put in the oven to dry at a temperature of 170^0C.For one hour. the samples were taken out of the oven and allowed to cool in the dessicator. The first weight was taken and recorded. The samples were then returned to the oven and subsequent weighing was done at 30 minutes interval. The process was repeated until a constant weight was obtained.

The percentage moisture content was then calculated thus

Weight of foil
        X grams

Weight of foil + macerated sample
        Y grams

Weight of macerated sample
            [Y – X] grams

Weight of dried sample + weight of foil
        Z grams

Weight of dried sample
        [Z – X] grams

% moisture content
        <u>loss in weight of sample</u> X 100

Weight of sample

        <u>[Z –X]grams – [Y – X] grams</u> X 100

[Y – X] grams (A.O.A.C 1990)

Titratable acidity determination

Five grams of each sample was weighed, macerated and transferred into a beaker containing 50ml of distilled water. The mixture was emulsified and left for about 10 minutes. 20ml of each food sample filtrates was then titrated against 0.1M NaOH with 2 drops of phenolphthalein indicator added. The end point was reached when there was a color change from colorless to pink. The titre value of each was read from the burette and recorded.

Ash content determination

An empty crucible was weighed for each of the food sample and the weight noted. Five grams of each sample weighed into already known weight of crucibles and were put in the furnace at 600^0C for 2 hours. The ash content was expressed as the ratio of the difference of the original weight of the samples and their weight after burning in the furnace to the original weight of the samples in percentage.

% moisture content = loss in weight of sample X 100

Original weight of sample

Microbiological analysis

Total Bacteria Count

The isolation and enumeration of the bacteria was done by using pour plate method. Fivefold serial dilution of the sample was prepared as follows; one gram of each macerated food sample was weighed aseptically and dispensed into 9ml of sterile distilled water to make 10^{-1} dilution. From this mixture with the use of sterile pipettes, 1ml was taken from 10^{-1} mixture into 9ml distilled water in a test tube to make 10^{-2} dilution rate. Further dilution was made up to 10^{-4}.One ml each was taken from 10^{-3} and 10^{-4} dilution using sterile pipettes and dispensed into sterile petri dishes. Cooled molten nutrient agar (NA) was poured aseptically and the plates were allowed to set. It was then incubated at 36^0C for 24 hours and examined for microbial growth.

Pure cultures were obtained by transferring a representative colony to a sterile solid nutrient agar surface and streaked using sterile inoculation loop. The plates were again incubated, it was continually subcultured until a satisfactory and desirable pure culture was obtained and was transferred into agar slants in MacCartney bottles and incubated for 24 hours and preserved in the refrigerator until it is needed.

Total fungal count

The isolation and enumeration of the fungi was done by using pour plate method. Five fold serial dilution of the sample was prepared by weighing one gram of each macerated food sample and dispensed into 9ml of sterile distilled water to make 10^{-1} dilution. From this mixture with the use of sterile pipettes, 1ml was taken from 10^{-1} mixture into another 9ml distilled water in a test tube to make 10^{-2} dilutionrate.1ml was also removed from 10^{-2} dilution with a sterile pipette into another 9ml sterile distilled water aseptically to make 10^{-3} dilution rate.1 ml from 10^{-3} was pipette

aseptically into sterile petri dishes and cooled molten potato dextrose agar (PDA) was poured on the sterile plates and the plates were allowed to set and incubated at room temperature of 25^0C for three to five days and examined for microbial growth. Different representative colonies were transferred to a sterile solidified potato dextrose agar using a sterile inoculating needle.

RESULT

pH values of the food samples

The pH values of the food samples are shown in table 1.0. For beans, it falls within the range of 5.57-6.40 for the first restaurant and 5.92-6.20 for the second restaurant. The pH of rice falls within the range of 5.88-6.09 for the first restaurant and 5.91-6.33 for the second restaurant. The pH of amala is within the range of 5.56-5.67 for the first restaurant and 5.56-6.0 for the second restaurant. The pH of eba is within the range of 5.81-6.06 for the first restaurant and 5.71-7.21 for the second restaurant.

Moisture content of the food samples

The moisture content of the food samples are shown in table 3.0. For beans, it ranges from 65%-70% for the first restaurant and 67%-68% for the second restaurant. In the case of rice, it falls within the range of 55%-60% for the first restaurant and 55%-50% for the second restaurant. The moisture content of amala falls within the range of 70%-72% for the first restaurant and 70%-72% for the second restaurant. For eba, the moisture content falls within the range of 65%-68% for the first restaurant and 65%-73% for the second restaurant.

Titratable acidity of the food samples

The titratable acidity for each food sample was determined. For beans, it ranges from 0.4ml/g-0.7ml/g for the first restaurant and 0.2ml/g-0.5ml/g for the second restaurant. For rice, it ranges from 0.5ml/g-0.6ml/g for the first restaurant and 0.6ml/g-0.7ml/g for the second restaurant. For amala, it ranges from 0.3ml/g-0.35ml/g for the first restaurant and 0.54ml/g-0.6ml/g for the second restaurant. For eba, it ranges from 0.5ml/g-0.6ml/g for the first restaurant and 0.3ml/g-0.4ml/g for the second restaurant.

Ash content of the food samples

The ash content for beans ranges from 3.0%-4.0% for the first restaurant and 3.0%-4.5% for the second restaurant. For rice, it ranges within 3.0%-4.0% for the first restaurant and 3.5%-3.7% for the second restaurant. For amala, it ranges within 1.0%-1.5% for the first restaurant and 1.5%-2.2% for the second restaurant. For eba, it ranges within 2.0%-3.0% for the first restaurant and 2.2%-2.9% for the second restaurant.

Table 1: Physicochemical properties of samples of food samples collected from restaurants in Tanke Oke-Odo, Ilorin.

Cooked Food samples	pH		% moisture content		% ash content		Titratable acidity(ml/g)	
	R1	R2	R1	R2	R1	R2	R1	R2
Beans	6.0 ± 0.29	6.1 ± 0.15	67.5 ± 2.5	67.5 ± 2.5	3.5 ± 0.5	3.5 ± 0.5	0.55 ± 0.02	0.35 ± 0.22
Rice	6.0 ± 0.11	6.1 ± 0.21	57.5 ± 2.5	52.5 ± 2.5	3.5 ± 0.5	2.7 ± 1.0	0.55 ± 0.02	0.65 ± 0.03
Amala	5.6 ± 0.10	5.8 ± 0.22	71.0 ± 1.0	71.0 ± 1.0	1.3 ± 0.3	1.9 ± 0.4	0.35 ± 0.02	0.55 ± 0.22
Eba	5.9 ± 0.13	6.5 ± 0.75	66.5 ± 1.5	69.0 ± 4.2	2.5 ± 0.5	2.5 ± 0.5	0.55 ± 0.22	0.35 ± 0.22

KEY: Values are mean of two readings with standard error mean
R1 represented the first restaurant.
R2 represented the second restaurant.

Total bacterial count (cfu/g)

The first restaurant had a total bacterial count ranging between 1.2 x 10⁴ cfu/g and 5.0 x 10³ cfu/g for beans. For rice, it ranged between 1.0 x 10⁴ cfu/g and 7.0 x 10³ cfu/g. For amala, it ranged between 1.1 x 10⁴ cfu/g and 9.0 x 10³ cfu/g. For ebaa, it ranges between 1.5 x 10⁴ cfu/g and 8.0 x 10³ cfu/g. The mean total bacterial count for beans was 8.5 x10³ cfu/g, for rice, it was 8.5 x 10³ cfu/g. For Amala, it was 1.0 x 10⁴ cfu/g and for Eba, it was 1.15 x 10⁴ cfu/g.

The second restaurant had a total bacterial count ranging between 2.1 x 10 ⁴ cfu/g and 2.0 x 10⁴ cfu/g for beans. For rice, it ranged between 2.2 x 10⁴ cfu/g and 1.3 x10⁴ cfu/g. For amala, it ranged between 1.7 x 10⁴ cfu/g and 1.6 x 10⁴ cfu/g and for Eba, it ranged between 1.9 x 10⁴ cfu/g and 1.5 x 10⁴ cfu/g. The mean total bacterial count for beans was 2.05 x 10⁴ cfu/g, for rice, it is 1.75 x 10⁴ cfu/g. For Amala, it was 1.65 x 10⁴ cfu/g and for Eba, it was 1.7 x 10⁴ cfu/g.

From the values above, the second restaurant had the higher mean bacterial count for all the food samples compared to the first restaurant. Total of five bacteria isolates were obtained and identified as *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Salmonella* sp., and *Shigella* sp.

Total fungal count (cfu/g)

The first restaurant had a total fungal count ranging between 3.0 x 10³ cfu/g and 1.0 x 10³ cfu/g for beans. For rice, it ranges between 2.0 x 10³ cfu/g and 6.0 x 10³ cfu/g. For amala, it ranges between 4.0 x 10³ cfu/g and 3.0 x 10³ cfu/g and for eba, it ranges between 5.0 x 10³ cfu/g and 2.0 x 10³ cfu/g. The mean total fungal count for the food samples are 2.0 x 10³ cfu/g, 4.0 x 10³ cfu/g, 3.5 x 10³ cfu/g and 3.5 x 10³ cfu/g for beans, rice, amala and eba respectively.

The second restaurant had a total fungal count ranging between 7.0 x 10³ cfu/g and 5.0 x 10³ cfu/g for beans. For rice, it ranges between 5.0 x 10³ cfu/g and 7.0 x 10³ cfu/g. For amala, it ranges between

8.0 x 10^3 cfu/g and 9.0 x 10^3 cfu/g and for eba, it ranges between 6.0 x 10^3 cfu/g and 8.0 x 10^3 cfu/g. The mean total fungal count for the food samples are 6.0 x 10^3 cfu/g, 6.0 x 10^3 cfu/g, 8.5 x 10^3 cfu/g and 7.0 x 10^3 cfu/g for beans, rice, amala and eba respectively.

From the values above, the second restaurant had the higher mean fungal count for all the food samples compared to the first restaurant. Total of three fungi were obtained and identified as *Aspergillus flavus, Saccharomyces cerevisiae* and *Mucor mucedo.* The details of their description are given and the structures of the isolated fungi are shown in figure 1, 2 and 3.

Table 2.0: Variation in total bacterial and fungal count of food samples in Tanke Oke-Odo, Ilorin.

Food samples	Total bacterial count cfu/g		Total fungal count cfu/g	
	R1	R2	R1	R2
Beans	8.50 x 10^3 $\pm$ 3.5	2.05 x 10^4 $\pm$ 3.5	2.0 x 10^3 $\pm$ 1.0	6.0 x 10^3 $\pm$ 1.0
Rice	8.50 x 10^3 $\pm$ 3.5	1.75 x 10^4 $\pm$ 4.5	4.0 x 10^3 $\pm$ 2.0	6.0 x 10^3 $\pm$ 1.0
Amala	1.00 x 10^4 $\pm$ 1.0	1.65 x 10^4 $\pm$ 0.5	3.5 x 10^3 $\pm$ 0.5	8.5 x 10^3 $\pm$ 0.5
Eba	1.15 x 10^4 $\pm$ 3.5	1.70 x 10^4 $\pm$ 2.0	3.5 x 10^3 $\pm$ 0.5	7.0 x 10^3 $\pm$ 1.0

Values are mean of two readings with standard error mean

KEY:
R1 represent the first restaurant.
R2 represent the second restaurant.

Table 3.0: Distribution of isolates among food samples from the restaurants in Tanke Oke-Odo, Ilorin.

BACTERIA	Beans		Rice		Amala		Eba	
	R1	R2	R1	R2	R1	R2	R1	R2
Staphylococcus aureus	+	+	+	+	+	+	+	+
Bacillus cereus	+	+	+	_	_	+	+	_
Escherichia coli	+	+	+	+	+	+	+	+
Salmonella sp.	_	+	_	_	_	+	+	+
Shigella sp.	+	_	_	+	+	_	_	+
FUNGI								
Aspergillus flavus	+	+	_	+	+	+	+	+
Mucor mucedo	+	+	_	+	+	_	+	+
Saccharomyces cerevisiae	+	+	_	+	+	_	+	+

KEY
R1: represented the first restaurant.
R2: represented the second restaurant.
+ : Present
- : Not Present

DISCUSSION

A total of five bacterial and three fungal isolates were characterized and identified. The presence of these organisms in the food samples may be due to various disposing factors. Microbial cells are significantly affected by the pH of foods because they apparently have no mechanism for adjusting internal pH (Frazier and Westhoff, 1995). The inherent pH of cooked foods varies, although most foods are neutral or acidic. Cooked foods with low pH values usually are not readily spoilt by bacteria and are most susceptible to spoilage by yeasts and molds (Frazier and Westhoff, 1995). The pH of the cooked food samples analysed is within the neutrophilic range (5.56 to 7.21. Fungi grow between the alkaline extreme to the acidic extreme.

The presence and availability of water also affect the ability of microorganisms to colonize food. Water availability measured in terms of water activity of a solution is 1/100 of the relative humidity of the solution (when expressed as a percentage), hence the water activity of the collected food samples ranges within 0.5 and 0.73.

The results of the assessment of the food samples have the following bacterial and fungal isolates, the Bacterial isolates are *Staphylococcus aureus, Bacillus cereus, Escherichia coli, Salmonella* sp., and *Shigella* sp. The fungal isolates are *Aspergillus flavus, Mucor mucedo* and *Saccharomyces cerevisiae*. The implications of the microorganisms isolated to human health are discussed below:

Molds produce mycotoxins, which are secondary metabolites that can cause acute or chronic diseases in humans when ingested from contaminated foods. Potential diseases include cancers and tumors in different organs (heart, liver, kidney, nerves), gastrointestinal disturbances, alteration of the immune system, and reproductive problems.

Aflatoxins are still recognized as the most important mycotoxins. They are synthesized by only a few *Aspergillus* species, of which *A. flavus* and *A. parasiticus* are the most problematic. Their presence in the food samples could be as a result of the favourable environmental condition for their growth. The expression of aflatoxin-related diseases is influenced by factors such as age, nutrition, sex, species and the possibility of concurrent exposure to other toxins. The main target organ in mammals is the liver, so aflatoxicosis is primarily a hepatic disease. Conditions increasing the likelihood of aflatoxicosis in humans include limited availability of food, environmental conditions that favor mold growth on foodstuffs, and lack of regulatory systems for aflatoxin monitoring and control (Machida and Gomi, 2010).

B. cereus is a normal soil inhabitant, Their presence in the food could be as a result of contamination by dust raised into the air during the food preparation or serving and is frequently isolated from a variety of foods, including vegetables, dairy products and meat. It causes a vomiting or diarrhea illness that is becoming increasingly important in the industrialized world. Some patients may experience both types of illness simultaneously. Some strains of the *B.*

cereus group are able to grow at refrigeration temperatures. These variants raise concern about the safety of cooked, refrigerated foods with an extended shelf life. *B. cereus* spores adhere to many surfaces and survive normal washing and disinfection (except for hypochlorite and UVC) procedures. *B. cereus* food borne illness is likely under-reported because of its relatively mild symptoms, which are of short duration (Granum, 2005).

For many years, *E. coli* was considered a commensal of human and animal intestinal tracts with low virulence potential. The presence of this organism could be as a result of the use of feacally polluted water for the food preparation. It is now known that many strains of *E. coli* act as pathogens, inducing serious gastrointestinal diseases and even death in humans (Smith and Fratamico, 2005).

Shigella species are members of the family Enterobacteriaceae and are Gram negative, non motile rods. Presence of *Shigella* like *E.coli* which is faecal oral in origin. Symptoms include mild to severe diarrhea with or without blood, fever and abdominal pain. Further complications of the disease may be seizures, toxic megacolon, reactive arthritis and hemolytic uremic syndrome. Transmission of the pathogen is by the fecal-oral route, commonly through food and water. The infectious dose ranges from 10-100 organisms. *Shigella* spp. have a sophisticated pathogenic mechanism to invade colonic epithelial cells of the host, man and higher primates, and the ability to multiply intracellularly and spread from cell to adjacent cell via actin polymerization. *Shigella* spp. is one of the leading causes of bacterial foodborne illnesses and can spread quickly within a population (Lampel, 2005).

Staphylococcus aureus is a common cause of bacterial foodborne disease worldwide. Its presence in the food samples could be as a result of the dirty and unkempt behavior of the food handlers. Symptoms include vomiting and diarrhea that occur shortly after ingestion of *S.aureus* toxin-contaminated food. The symptoms arise from ingestion of preformed enterotoxin, which accounts for the short incubation time. Staphylococcal enterotoxins are superantigens and, as such, have adverse effects on the immune system (Stewart, 2008).

There are various factors which contributes to the presence of microorganisms in cooked food samples. Some of the factors include the location of the restaurants, the sanitary procedures of the restaurants, the source of water used in the preparation of food, the cooking utensils used, the food handlers, etc. It was observed that the first restaurant from which food samples was collected was situated very close to bushes and food handlers looked unkempt and dirty. In general, the environmental conditions and the sanitary procedures employed in the second restaurant was poorer compared to that of the first restaurant. Hence, the lower microbial count of food samples collected from the first restaurant.

CONCLUSION

Food is very important to the survival of human. It supplies nutrients to the body for effective functioning and metabolism. Spores of microorganisms when heated

with foods germinate and are not all destroyed. Foods that have been heated to destroy the competing microflora and given prolonged storage at room temperature should be reheated before consumption to destroy the toxins which are thermolabile avoid intoxication.

To reduce the outbreak of diseases that results from the consumption of ready to eat food, all the measures discussed earlier should be taken into consideration. Proper cooking of food and proper storage should be done to prevent the contamination and growth of microorganisms in food so as to eliminate the risk of infection or intoxication.

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