Microbiological qualityof street-vended ready-to-eat Ekpainkpoto (plantain pudding) in Bayelsa state, Nigeria

ABSTRACT

Maintaining good microbiological quality of ready-to-eat foods is an essential element in ensuring the protection of the public from food-borne diseases that can be transmitted via contamination of these foods. This study was thus focused on assessing the microbiological quality of plantain pudding (ekpainkpoto) sold in markets in Bayelsa State. The study adopted a cross-sectional design involving 50 samples of plantain pudding obtained from different markets in Bayelsa state. The samples were collected in sterile polyethylene bags and sent for microbiological analysis within 24 hours of collection. Seven bacterial isolates were identified after analysis which included *Bacillus* sp. (20.3%), *Staphylococcus* sp. (17.5%), *Lactobacillus* sp. (17.5%), *Proteus* sp. (15.3%), *Streptococcus* sp. (13.6%), *Micrococcus* sp. (10.2%), and *Escherichia coli* (7.8%). A total of five fungal isolates were detected and isolated as follows *Candida* sp. (17.0%), *Penicillium* sp. (25.0%), *Aspergillus* sp. (29.0%), *Fusarium* sp. (29.0%) and *Rhizopus* sp. (17.0%). Total viable counts of bacterial and fungal isolates (cfu/g) in the plantain pudding samples revealed that *Escherichia coli* (bacterial) and *Rhizopus* sp. (fungal) respectively had the highest total viable counts of 8.0 $x10^9$ cfu/g apiece. The laboratory assessment of the ekpainkpoto samples revealed that they all had unacceptable levels of bacterial and fungal contamination. It was recommended that health education of the food vendors on safe food handling as well as regular monitoring action by regulatory bodies be ensured to improve the quality of ekpainkpoto and other ready-to-eat foods.

Key words: Ready to eat foods, plantain pudding, microbiological quality, Bayelsa

Introduction

Plantain (*Musa sapientum var. Paradisiacal Linn*) is astaple food widely eaten in different parts of the worldincluding in Nigeria, where its consumption cuts across multiethnic and socioeconomic divides. It can be consumed in its roasted form (bole) or boiled and eaten with vegetable soup or stew(Obinna-Echem & Eze, 2020). It can also be fried into chips, pounded, or steamed, while overripe ones can beprocessed into beer or utilized in the preparation ofsome cereal and legume pudding, locally known as *"Ekpainkpoto"*(Obinna-Echem & Eze, 2020; Okojie & Isah, 2014).It is a widely consumed food in Southern Nigeria and can also be prepared by mixing ripe and unripe plantain powder to produce the plantain pudding which is usually sold as a ready-to-eat food on the streets. This implies that they are available for

immediateconsumption at the point of sale withoutfurther treatment(Oluwafemi et al., 2013; Oranusi & Braide, 2012).

Street foods like plantain pudding, roasted plantain and so on, are well patronized and form an integral part of the food supply chain in southern Nigeria considering their easy accessibility and ability to serve as asource of income(Eke-Ejiofor & Maxwell, 2019; Okojie & Isah, 2014).These notwithstanding, street vended plantain and foods in general, largely do not meet proper hygienic standards. They can thus lead to food-borne morbidities, outbreaks and/or mortalities(Eke-Ejiofor & Maxwell, 2019; Oluwafemi et al., 2013). The contamination of these type of readily-available foodsby microorganisms could occur during handling,storage and transportation of the foods, usually as a result of unhygienic as well as improper processing and storage conditions(Ayoade & Adegbite, 2016; Eke-Ejiofor & Maxwell, 2019).This could be from contamination of: cooking utensils used for preparation, water for mixing, knife for peeling, the mixing equipment, as well as contamination from sneezing and coughing over the foods by the handlers(Oluwafemi et al., 2013).

The vendors of these foods could be stationary or migratory. Stationary vendors sell their wares fromsmall stalls, kiosks or pushcarts, and operate fromstrategic locations, such as bus and trains,markets, commercial districts,outside schools, residential and so on. Migratory food vendors on the other hand move from one location to the other and cannot be directly confronted in the event of the production of a bad product(Oranusi & Braide, 2012).These migratory vendors have nofixed location or routes on highways, and their movement could be dependenton newly identified failed portions on the highways,repair/construction works as well as security posts on the highways. It is also possible that they can move about different locations/routes todeliberately sell bad or unhygienically prepared food products(Oranusi & Braide, 2012).This poses a source of public health concern considering that in the event of morbidities or outbreaks, they are not easily traceable, thus making containment difficult(Eke-Ejiofor & Maxwell, 2019; Oranusi & Braide, 2012).In Nigeria, this problem is worsened by issues of inadequate supervision and proper monitoring by food safety officers, weak enforcement of food hygiene regulations, lack of training in food safety and good hygiene practices, unavailability of potable or running water at vendingsites, and so on(Okojie & Isah, 2014). Considering that the contamination of these plantain-based, ready-to-eat foods are a reality but not widely researched, it is essential to provide more evidence to strengthen the course for advocating improved hygienic standards in the production and sale of these foods. It thus became necessary to determine the microbiological quality of Ekpainkpoto (plantain pudding) sold in Bayelsa state, Nigeria.

Materials and Methods

This study adopted a descriptive design to assess the microbiological quality of 50 plantain pudding samples obtained from different markets in Bayelsa state by convenience sampling. The samples were collected in sterile polyethylene bags and taken to the laboratory for microbiological analysis within 24 hours of collection.

Media Preparation

All media and reagents used were prepared according to the manufacturer's directions. The media used were Nutrient agar, Potato dextrose agar and de Man Rogosa.

Microbiological Analysis

One gram of plantain pudding was dissolved in 100ml of distilled water and homogenized in a conical flask. One (1ml) of the mixture was then serially diluted. Then 0.1ml of the sample was inoculated into nutrient agar, to assess for bacterial growth, McConkey agar as well as de Man Rogosaand Sharpe agar (MRSA) to assess for lactic acid bacterial growth and potato dextrose agar to assess for fungal isolates.

Enumeration of Bacteria Isolates

Bacteria count was done on nutrient agar plates after inoculation at 37^{o}C for 24 hours. The total numbers of identified colonies were counted and expressed as colony forming unit per gram (cfy/g).Pure cultures of the mixed bacteria population were obtained by sub-culturing onto sterile nutrient agar, McConkey agar, and de Man Rogosa and Sharpe agar (MRSA). Plates were then incubated at 37^{o}C for 24 hours. Colonies obtained from these cultures were preserved on agar slants at 4^{o}C(Cheesbrough, 2006).

Gram Staining

The gram staining was done following the steps below:

- Using a sterile wire loop, a smear of the colonies was made on clean grease-free slides.
- The bacteria were then fixed to the slide by flaming and sometimes by allowing them to air dry.
- The fixed smear was covered with crystal violet (primary stain) for 60 seconds.
- Lugol's iodine (a mordent) was added to the stain and allowed to stay for another 60 seconds.
- This was then decolorized with acetone for 10 – 30 seconds and washed with excess water.
- The stained smear was then counterstained with neutral red and allowed to stay for 30 seconds and then washed with excess water.

- The stained slides were thereafter allowed to air-dry, after which they were viewed under the microscope using the 100x objectives with oil immersion. The gram-positive cells were recorded as purple colored cell while gram negative cells appeared at pink colored cells(Cheesbrough, 2006).

Enumeration of Fungi

Detection of fungi was done on potato dextrose agar after incubation at 28^{o}C for two to three days.Culturecharacteristics such as colour, morphology and mycelia growth patterns were checked. During microscopy,phenol cotton blue was placed on the clean slide, and a small quantity of the isolate was removed and transferred to the stain on the slide and an inoculating needle was used to tease out the sample carefully and covered with a cover slide to prevent air bubbles.Once these were done, viewing was done to identify relevant characteristics.

Spore Staining

The test was performed as described by Chi and Kolhalkar, (2000). Each isolate was streaked onto nutrient agar plates and inoculated at 37^{O}C for 5 days. A smear of each isolate was made on clean, dry, grease-free slide and allowed to air dry. The slide was placed on the rim of beakers containing water heated over a Bunsen flame, so that rising water vapour heated the underside of the slide. The smear was flooded with malachite green solution and further heated for one minute. The slide was then washed in gentle running tap water and counter stained with 0.5 (w/v) Safranin solutions for 30 seconds.This was then washed, dried and viewed under oil immersion (x100) of the light microscope, where spores stained green while vegetative cells, stained red(Chi & Kolhtkar, 2000).

Biochemical Testing

Catalase Test

A clean glass slide was flooded with a drop of normal saline. Loopfuls of the test isolates were used to make a smear on the glass slides with a sterile wire loop. A drop of hydrogen peroxide was then added onto the slide and examined. The absence of bubbles showed catalase-negative microorganisms.

Indole Test

The test isolates were grown in peptone water for forty-eight hours at 37^{o}C. Thereafter five drops of Kovsc's reagent were added to the medium.Red coloration of the medium indicated indole-positive microorganisms.

Urease Test

Loopfuls of the test isolates were inoculated onto Christensens urea agar and incubated at 37^{o}C for twenty-four hours. Pink coloration of the medium indicated urease-positive organisms.

Triple Sugar Iron (TSI)

A sterile straight wire loop containing the test isolates was used to stab the medium and streaked in a zigzag pattern. Blackening along the line of the stab indicated hydrogen sulphide production(Cheesbrough, 2006).

Oxidase Test

This test was used to identify microorganisms that have the cytochrome oxidase enzyme. Oxidase reagent was prepared by weighing one gram of the solid 100ml distilled water and strips Whatmanfilter paper was soaked in the solution.Loopfuls of the test isolates were then placed on the filter paper. The formation of dark-purple coloration indicated the presence of oxidase positive organisms.

Coagulase Test

This test is used to identify microorganisms that have coagulase enzymes. A drop of physiological saline was placed on each end of the slide to make a thick suspension. A drop of plasma was added to make one of the suspensions and mixed gently. Clumping of the cells within ten seconds indicated a positive test.

Methyl Red / Voges – Proskauer (MR/VP)

The test isolates were inoculated into MR/VP media and incubated at 37^{o}C for 48 hours. Five drops of methyl red were added to the test isolates and shaken gently. The formation of pink coloration indicated a positive test.

Carbohydrate Utilization Test

This test assessed an organism's ability to ferment glucose as well as its ability to convert the end product of glycolysis (pyruvic acid) into gaseous by-products. One litre of peptone was prepared by dissolving 10g of peptone and 5g of sodium chlorine in 100ml of distilled water with a phenol red as a pH indicator. 9ml of the peptone water was dispensed into test tubes with inverted Durham's tubes and sterilized by autoclaving at 121^{o}C for 15min at 15psi. 10% of the glucose solution was prepared by dissolving 10g in 100ml of distilled water and sterilized by autoclaving at 121^{o}C for 8 minutes at 15psi. 1ml of the sugar solution was aseptically added to 9mls of the sterile peptone water. The medium was incubated with the test isolates and incubated at 37^{o}C for 24 – 48 hours. The formation of a bubble at the top of the test tubes indicated positive isolates(Cheesbrough, 2006).

Results

The result of percentage occurrence of bacteria isolate in the plantain samples is shown in Figure 1. They include*Staphylococcus aureus*(17.5%), *Bacillus* sp (20.3%), *Streptococcus* sp. (13.6%), *Escherichia coli* (7.8%), *Micrococcus* sp (10.2%), *Lactobacillus* sp. (17.5%), *Proteus* sp (14.2%). Figure 2 also shows the percentage occurrence of fungi isolates in plantain pudding of which *Aspergillus* sp. and *Fusarium*sp. has the highest percentage occurrence of 29.0%, *Penicilum* (25.0%) respectively.

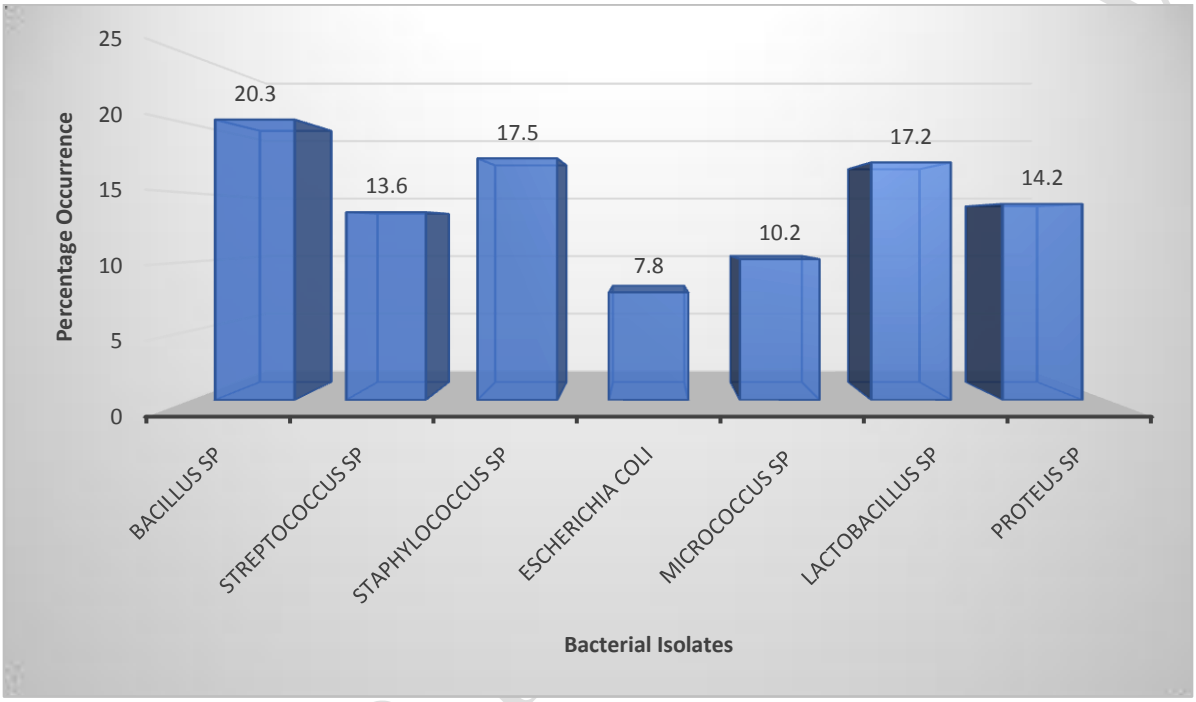

Figure 1: The percentage occurrence of bacteria isolates in plantain pudding

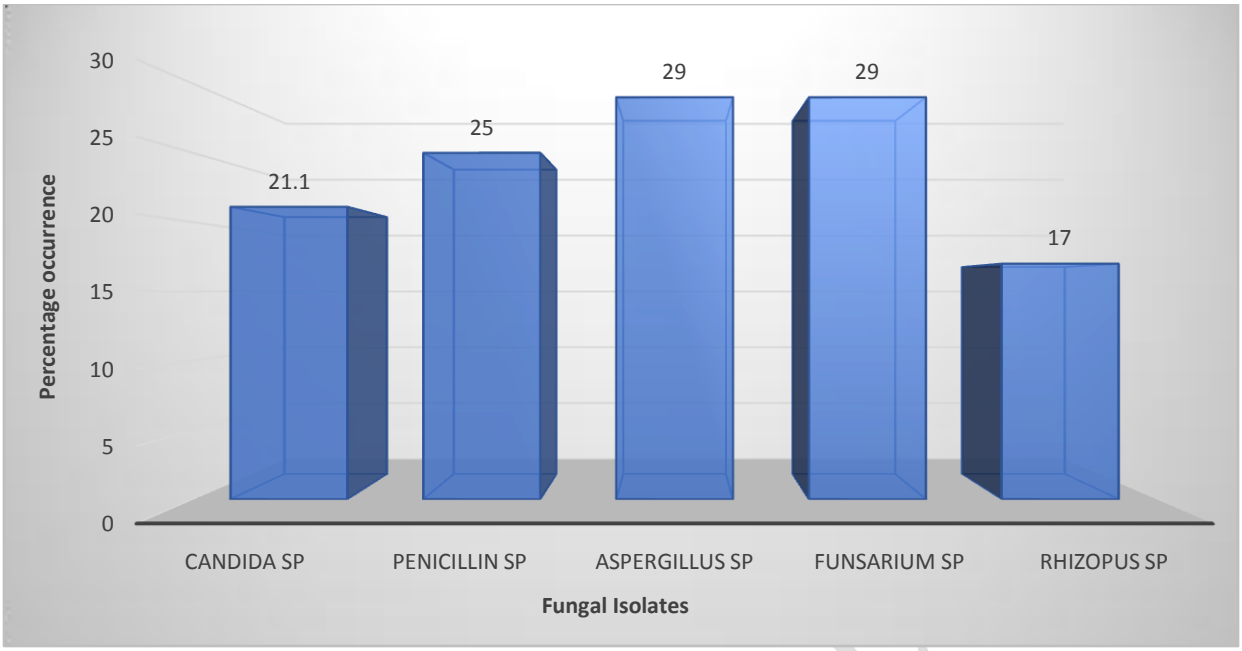

Figure2: Percentage occurrence of fungi isolate in plantain pudding

As seen in Tables 1and 2, total viable counts of bacteria and fungal isolates (cfu/g) respectively in the plantain pudding samples revealed that *Escherichia coli* (bacterial) and *Rhizopus* sp. (fungal) had the highest total viable counts of 8.0 $x10^9$cfu/g apiece.The tables also show the presence of other contaminating florain the plantain pudding samples.

Table 1: Total viable counts of bacteria isolate (cfu/g) in plantain pudding

Organisms	**Viable counts (cfu/g)**
Bacillus sp	$2.2x10^9$
Streptococcus sp	$1.6x10^9$
Staphylococcus sp	$2.0x10^9$
Escherichia coli	$8.0x10^9$
Micrococcus sp	$1.2x10^9$
Lactobacillus sp	$2.0x10^9$
Proteus sp	$1.8x10^9$

Acceptable levels: 0-10^3, Tolerable levels: 10^4- ≤10^5, Unacceptable: 10^6 and above(Oranusi et al., 2013)

Table 2: Total viable counts offungi isolates (cfu/g) in plantain pudding

Fungi Isolates	Percentage occurrence in food
Candida sp	$1.0 x10^9$
Penicillin sp	$1.2 x10^9$
*Aspergillus*sp	$1.4 x10^9$
*Funsarium*sp	$4.0 x10^9$
*Rhizopus*sp	$8.0 x10^9$

Acceptable levels: 0-10^3, Tolerable levels: 10^4- ≤ 10^5, Unacceptable: 10^6 and above(Oranusi et al., 2013)

Discussion

This study has revealed that plantain pudding (ekpainkpoto) samples analyzed for microbiological safety, were contaminated by unacceptable levels of bacterial and fungal isolates. This assessment was based on the prescribed standards by regulatory authorities for these microbes in ready-to-eat foods(Food and Agricultural Organization & World Health Organization, 2005; Oranusi et al., 2013). The finding of bacterial and fungal isolates in a ready-to-eat food (ekpainkpoto) assessed in this study is similar to the findings of other authors who assessed the microbiological quality of other related and unrelated ready-to-eat foods. These included the study that identified high microbiological contaminant levels in roasted plantain in Rivers State(Eke-Ejiofor & Maxwell, 2019), high levels in coleslaw(Oranusi et al., 2013)as well as similar contamination of street-vended foods and ready-to-eat vegetables in Southwestern Nigeria,(Oluwafemi et al., 2013), and in Port Harcourt metropolis(Oyet et al., 2020). These studies also presented with similar contaminants identified in the present study including Staphylococcal contaminants, Bacillus sp., Escherichia coli as well as fungal isolates(Eke-Ejiofor & Maxwell, 2019; Oranusi et al., 2013; Oyet et al., 2020).

The implication of these findings is that the presence of these bacterial and fungal contaminants in the ready-to-eat pudding could be related with the release of harmful microbial toxins related with the occurrence of disease conditions in human populations(Obinna-Echem & Eze, 2020). The findings also provide an indication of various contaminant sources which these ready-to-eat foods were exposed to during their preparation and processing. The presence of *staphylococcus aureus,* for exampleis an indication of respiratory contamination during processingby the persons involved inselling the plantain pudding. It is also a pointer of excessive human contact with the processed plantain pudding considering that this microbe is a natural body flora(Eke-Ejiofor & Maxwell, 2019). Also, the presence of Escherichia coli (an indicator of fecal contamination) in the evaluated food is indicative of the lack of hygiene in handling the food as well as the use of water of poor quality for the preparation of the food(Eke-Ejiofor & Maxwell, 2019). The presence of the various contaminants could also be an indication of unhygienic conditions related to the location of the stalls where the plantain pudding is sold, especially along dusty or dirty roadsides, as well as unhygienic surroundings(Canini et al., 2013; Okojie & Isah, 2014; Oyet et al., 2020).These contaminants could have also prevailed as a result of the maintaining the ekpainkpoto at a temperature that allowed the proliferation of these microbes as well as the production of their toxins. It is also possible that contamination could have occurred due to the use of unclean hands (that may have been used for handling money, touching clothing, processing raw materials) for packaging the pudding(Obinna-Echem & Eze, 2020).It is important to note that the handling of these foods with contaminated hands by the food vendors could be as a result of their lack of awareness of the dangers of handling ready-to-eat foods with hands that have not been hygienically cared for(Oranusi & Braide, 2012).

In light of these potential sources of exposure to contaminants, it is recommended that improving food vendors awareness and practice of hygienic processing, packaging and sales of the plantain pudding (ekpainkpoto) is an effective measure that can be undertaken to improve the microbiological quality of the food.This is especially important considering the large proportions of persons who patronize street vendors to obtain ready-to-eat foods such as plantain pudding (Obinna-Echem& Eze, 2020; Okojie & Isah, 2014).They can also be enlightened on the need for maintaining a hygienic environment during the preparation of the food. In addition, this health education initiative can be conducted in collaboration among the food vendors, local authorities, government agencies, as well as standards'setting bodies. It is also imperative that regular monitoring and sampling for microbial quality of the pudding be done by the necessary regulatory agencies and ministries of government.

Conclusion

This study has shown that samples of plantain pudding (ekpainkpoto) obtained from different markets in Bayelsa State were contaminated with bacterial and fungal contaminants beyond acceptable standards for microbiological quality of ready-to-eat foods.This study has been able to show that contamination of plantain pudding sold in Bayelsa State is a possibility which could have been from handling and processing procedures associated with its preparation.

This study has a limitation with respect to the descriptive design used to approach a topic that could have been approached better using an analytic design considering the public importance of the study focus.This notwithstanding, this study has provided insight into food safety issues that could be related with the consumption of street-vended ready-to-eat plantain ppudding in Bayelsa State.Analytic studies are thusneeded to identify specific points of exposure to contamination of plantain pudding sold in Bayelsa State as well as if any association exists between the educational status of food vendors and the practice of hygiene during the preparation of ekpainkpoto in Bayelsa State.

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