

Bacteriological Quality of Commercially Sold Fried Fermented Cereal (Masa) in Wukari, Nigeria.

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ABSTRACT

The bacteriological quality of fried fermented cereal (masa) sold in Wukari metropolis was evaluated. A total of 9 samples, three from each location, opposite school Federal University Wukari, Taraba State, Takum junction and Yam market and laboratory prepared sample (control) were evaluated for bacteria load and the presence of bacteria using standard microbiological techniques. The result showed no bacteria growth on the laboratory prepared masa. The total bacteria count of the commercial samples ranged from 1.0×10^6 cfu/g to 6.0×10^7 cfu/g. Total coliform count of 3.4×10^7 cfu/g was only observed in sample OPS1. There was no lactic acid bacteria growth. The bacteria isolated from the various samples and their occurrence showed that *Staphylococcus* species was the highest (60%), followed by *Enterococcus* species (30%) and *Pseudomonas* species was the least (10%). The high bacteria count and the presence of potential pathogenic bacteria in some of the sample is an indication that the masa samples were contaminated and this could potentially pose health hazard to the consumers. Hence, personal and environmental hygiene as well as good manufacturing practices are required during production of masa to avoid food borne diseases outbreak as a result of food contamination with potential pathogens encountered in this study.

Keywords: Masa, fermentation, contamination, health hazards, hygiene

INTRODUCTION

Masa is a traditionally fermented snack that is usually prepared and consumed in the Northern and some Western States in Nigeria (Maiangwa *et al.*, 2013). Masa is also referred to as "WAINA" and can be defined as a 'Nigeria yeast fermented puff -batter of cereals cooked in a pan with individual cup-like depression' (Ayo *et al.*, 2008). It is seen as a traditional food that is consumed by all class of ethnic divisions in Nigeria. Masa is consumed as a staple in Northern Nigeria as well as other African Countries including Niger, Mali, Ghana, and Burkina-Faso (Maiangwa *et al.*, 2013). Masa can be produced from a variety of cereals such as rice, maize, sorghum, and millet which helps to define the types of masa produced. For instance, masa shinkafa from rice, masa masara from maize, masa gero from sorghum, and masa dawa from millet (Badau *et al.*, 2018).

Good quality Masa is round in shape with brown color and smooth surfaces. The brown crisp edges and the mild sour taste are considered by many consumers as the quality masa attribute required of Masa (Ayo *et al.*, 2014). The consumption of masa brings about some certain health benefit to consumers because it contains high

calories and calcium minerals. It is also a good source of energy and fibre which helps to control appetite and prevent constipation (Badau *et al.*, 2018). Masa serves as a breakfast and snack for many people (Ayo *et al.*, 2008). It is eaten both casually and ceremonially and can be eaten with granulated sugar or with honey because of its sour taste due to its high acidity (Sanni and Adesulu, 2013).

As a fermented food, Masa is processed through spontaneous fermentation of the cereal grains or introduction of a starter culture to initiate the fermentation process. Baker's yeast is usually used as the commercial starter (Maiangwa *et al.*, 2013). Fermentation is known to soften food texture and alter its composition in such a way that it will require minimal energy both in the cooking and preservation process (Badau *et al.*, 2018). It is one of the important techniques employed to extend the shelf life of raw food materials, to develop and add aroma to a variety of diets (Achi, 2005).

The processing of cereals is carried out using rudimentary equipment and often in an unhygienic environment especially during processing and handling. Microbes are known to be ubiquitous; therefore, the products could be prone to unintentional contaminations by microorganisms found in the environment and also contaminated water used for the production (Badau *et al.*, 2018). The vendors' practices poor personal hygiene and reports of food vendors being carriers could therefore serve as a potential source of enteric fevers are many. Most of them have had either no formal education or few years of schooling and therefore lack knowledge on proper food handling and their roles in transmission of pathogens (Yeboah-manu *et al.*, 2010). Sometimes these foods are held at temperatures that are not proper and excessively handled by the food vendor and sold at very dirty environment (Ghosh *et al.*, 2007).

Therefore, there is need to conduct the bacteriological quality of Masa that is sold within different locations in Wukari. The bacteriological quality of food indicates the amount of bacteria contaminants the food has; a high level of contamination indicates low quality and more likely to transmit disease while low contamination indicates a good quality and safe food and is less likely to transmit disease (Yeboah-manu *et al.*, 2010).

MATERIALS AND METHODS

Sample Collection

A total of 9 samples were collected aseptically in sample bags from three selected vendors in 3 different locations namely: Opposite school (OPS) of Federal University Wukari, Taraba state, Takum junction (TJ) and yam market (YM) in Wukari Local Government Area, Wukari, Taraba state. Three masa samples each from the vendors were collected and labeled properly. A laboratory sample was also prepared from rice, sugar, yeast, baking powder, dried pepper and onions purchased from Wukari new market as control. The collected samples are taken to the Biology laboratory of Federal University Wukari for analysis. Samples were stored in the refrigerator at 4°C for further use.

Enumeration of Bacteria

The bacteriological analysis of masa samples was done to determine the bacteria load and presence of bacteria in the various samples using the method described by (Ogodo *et al.*, 2018). One (1) gram of each sample and control was weighed and

dispensed into 9ml of sterile distilled water and serially diluted up to 10^{-5} . Serial dilution was conducted by adding 1ml from tube one into the second test tube and mixed carefully by shaking it gently. The same procedure was made several times. Pour plate technique was used for plating the Masa dilution. Sterile petri-dishes were labeled corresponding to the number of tube containing the dilution. Separate sterile string was used to transfer 0.2ml of sample from the dilution tube to the corresponding petri-dishes before pouring molten agar that has been cooled to 45°C after sterilization. Nutrient agar (NA) for total bacteria count, MacConkey agar (MCA) for total coliform count and De Man Rogosa and Sharpe agar (MRS) for total lactic acid bacteria count. This was swirled gently to mix the agar and allowed to solidify before it was incubated at 37°C for 24hours while De Man Rogosa and Sharpe agar (MRS) plates were incubated anaerobically in an anaerobic jar. The total plate count for the growth of bacterial colonies on plates was observed using colony counter to count and record the number of colonies on each plate. The actual number of bacterial colonies was calculated as colony forming unit (CFU/g) (Badau *et al.*, 2018). Which is calculated using the total number of colonies counted divided by the volume of inoculum multiplied by the dilution factor. That is;

$$\text{CFU/g} = \frac{N}{V \times D}$$

Where;

CFU/g= Colony forming unit per gram of weighed sample.

N= Total number of colonies counted

V= Volume of inoculum

D= Dilution factor

Isolation and Identification of Bacteria

After 24 hours of incubation, plates were examined for bacteria growth and then counted. Distinct colonies were then sub-cultured into freshly prepared agar medium and incubated at 37°C for 24hours to obtain pure cultures after successive streaking. The pure cultures were subjected to the four-way identification techniques: Macroscopy (to ascertain the cultural characteristics such as size of growth, edge, elevation, pigmentation etc), Microscopy (to determine the cell morphology and arrangement after Gram staining), biochemical tests (such as catalase, oxidase, indole, citrate coagulase etc.) and sugar fermentation tests using the methods described by Okereke and Kanu (2004) and with reference to Badau *et al.* (2018).

RESULTS

Table 1 shows the total bacterial count on nutrient agar which ranges from 1.0×10^6 cfu/g to 6.0×10^7 cfu/g. In all the samples opposite school Federal University Wukari, Taraba state sample 1 has the highest total bacterial count with the least in opposite school sample 3. The total bacterial count on MacConkey agar was 3.4×10^7 cfu/g found in opposite school sample 1. There was no growth on De Man Rogosa and Sharpe agar.

Table 2 shows bacteria isolated from the samples and their identification. The result shows that the isolates are *Staphylococcus* species, *Enterococcus* species and *Pseudomonas* species.

Table 3 presents the distribution of the isolates on the samples. The results showed that *Staphylococcus* species was present in OPS1, OPS2, YM2, YM3 and TJ3,

Pseudomonas species was present in OPS1 while *Enterococcus* species was present in OPS1, OPS2 and TJ3.

Table 4 shows the occurrence of the isolates on masa. A total of 10 samples were examined and *Staphylococcus* species, *Pseudomonas* species and *Enterococcus* species were isolated respectively and have a percentage occurrence of 60%, 10% and 30% respectively in the examined samples.

Table 1: Mean microbial load of masa samples

Sample code	Total bacterial count(cfu/g) in nutrient agar (NA)	Total bacterial count(cfu/g) in MacConkey agar(MCA)	Total bacterial count(cfu/g) in De man Rogosa and Sharpe agar(MRS)
OPS1	6.0×10^7	3.4×10^7	-
OPS2	7.5×10^6	-	-
OPS3	1.0×10^6	-	-
TJ1	-	-	-
TJ2	12.5×10^6	-	-
TJ3	3.0×10^6	-	-
YM1	3.0×10^6	-	-
YM2	7.5×10^6	-	-
YM3	1.5×10^6	-	-
Control	-	-	-

Keys: - =no growth, OPS= opposite school Federal University Wukari, TJ =Takum junction and YM =yam market, 1-3 is the different collection point at a location.

Table 2: Bacteria isolated from masa and their identification

S/ N	Morphological characteristic	MICROSCOPY		BIOCHEMICAL TEST			SUGAR FERMENTATION TEST			Organism	
		Gram's reactio n	Cell arrangeme nt	Catalas e	Oxidase	Indole	Citrate	Gluco se	Sucro se		Lacto se
1	Large and small, spherical, creamy, and slightly raised colonies.	+	Cocci in clusters	+ve	+ve	-ve	+ve	+ve	+ve	+ve	Staphylococcus species
2	Red-brown colonies with entire edges.	-	Rod in clusters	+ve	+ve	-ve	+ve	-ve	-ve	-ve	Pseudomonas species
3	Smooth, white colonies with entire edges	+	Cocci in clusters	-ve	-ve	-ve	-ve	+ve	+ve	+ve	Enterococcus species

Keys: +ve=positive results, -ve=negative results.

Table 3: Distribution of bacteria isolated in the masa samples.

Sample code	<i>Staphylococcus</i> species.	<i>Pseudomonas</i> species	<i>Enterococcus</i> species
OPS1	+ve	+ve	+ve
OPS2	+ve	-ve	+ve
OPS3	-ve	-ve	-ve
YM1	-ve	-ve	-ve
YM2	+ve	-ve	-ve
YM3	+ve	-ve	-ve
TJ1	+ve	-ve	-ve
TJ2	-ve	-ve	-ve
TJ3	+ve	-ve	+ve
C	-ve	-ve	-ve

Keys: +ve=positive results, -ve=negative results,OPS=opposite school Federal University Wukari, TJ=Takum junction and YM=yam market. (1-3 refers to the sample collected at a location).

Table 4: Occurrence of the isolates on the samples

Isolates	No examined	No of positive	% occurrence
<i>Staphylococcus</i> species	10	6	60
<i>Pseudomonas</i> species	10	1	10
<i>Enterococcus</i> species	10	3	30

DISCUSSION

Pathogenic bacteria are the most commonly known causes of food contamination and food borne illness. This study aimed to analyze the bacteriological quality of a fried fermented cereal (masa) sold in Wukari. Masa is described as fermented puff batter made from rice, millet, maize or sorghum that is traditionally cooked in pan with individual cup-like depression. Masa is a traditionally fermented snack that is usually prepared and consumed in the Northern and some Western States in Nigeria. It is seen as a traditional food that is consumed by all class of ethnic divisions in Nigeria. However, it can be a source of disease transmission due to contamination by pathogenic microorganisms during preparation, storage and marketing.

A total of 9 samples of fried fermented cereal (masa) were collected and examined with a laboratory prepared sample for bacteria load and type of bacteria associated with them. In the present study, there was no bacteria growth in the laboratory prepared masa. This could be due to aseptic techniques used in the preparation of the laboratory prepared masa sample. The bacteria count for commercial masa show that the total bacteria count ranged from 1.0×10^6 cfu/g to 6.0×10^7 cfu/g. The count were quite high, however, similar count ranging from 4.26×10^2 cfu/g to 8.33×10^2 cfu/g has been reported on masa samples (Badau *et al.*, 2018). The present study has a higher bacteria count compared to the report of Badau *et al.* 2018 in Maiduguri, Borno State of Nigeria. The high bacteria count can be as a result of contamination of the utensils used during processing and the hygiene of the producer. It can also be as a result of contamination from the environment as well as processing equipment and water.

The coliform count in the commercial sample was 3.4×10^7 cfu/g observed in opposite school sample 1 only. This could be as a result of poor personal hygiene, improper sewage disposal or faecal contamination. Coliform bacteria are seen as indicators of some degree of potentially hazardous faecal contamination. This can also be associated with contamination during processing, storage and handling, especially from water used for processing which can probably be due to inadequate water supply in the study area (Ogodo *et al.*, 2018).

The occurrences of the bacteria showed that *Staphylococcus* species were present in sample OPS1, OPS2, YM2, YM3, TJ1, and TJ3, *Pseudomonas* species were present in only OPS1 samples and *Enterococcus* species were present in OPS1, OPS2 and TJ3 samples. The occurrence of these isolates showed that *Staphylococcus* species was the most common organism in the sample 6(60%), followed by *Enterococcus* species 3(30%) and the least was *Pseudomonas* species 1(10%). This can be as a result of contaminants from personnel through dirty hands, clothes, and utensils that come in contact with the food.

The presence of *Staphylococcus* species has been reported to indicate environmental and human contaminations. *Staphylococcus* species are common organisms found in individuals and are expelled from the respiratory tract, nose, mouth, clothing, and hands (Badau *et al.*, 2018). The presence of the toxins can cause serious gastroenteritis. The presences of *Pseudomonas* species which are opportunistic pathogens are known to cause food spoilage and lead to economic loss. The fried fermented cereal (masa) is sold with stew or other soups or grinded pepper which can also increase the level of contamination; this is because of water content in the soups or stew and the pepper which is not cooked and may be left over (Yeboah-Manu *et al.*, 2010).

The result showed that the fried fermented cereal (masa) can be a source of food borne disease transmission if the load exceed the acceptable limit for ready to eat foods. This study is in agreement with the findings of Badau *et al.* 2018 on the microbial quality evaluation of masa processed and sold within University of Maiduguri campus, Borno state.

CONCLUSION

The analysis of Masa for the isolation and enumeration of bacteria has shown that there are bacteria contaminants in Masa which may be due unhygienic processing and poor handling practices, use of contaminated water, and dirty utensils, which may potentially serve sources of pathogens and can lead to public health issues when consumed. Hence there is need for hygienic measures during preparation and storage of masa to avoid the outbreak of infections from the organisms encountered in this study.

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