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Microbial quality evaluation of *masa* processed and sold within University of Maiduguri campus

Abstract

Masa is a fermented bread-like product, which is round in shape with brown colour and smooth surface made from millet, maize, or rice flour. The total bacterial aerobic plate count, coliforms, mould, yeast and staphylococcal counts of *Masa* were determined. Discrete colonies appearing on incubated plates were isolated and identified. The results showed that South-east of University area had the highest bacterial counts, ranging from $4.26x10^2$ to $8.33x10^2$CFU/g and coliforms counts ($1.331x10^2$ to $3.14x10^2$CFU/g). North-east sample had the highest yeast count ($3.2x10^2$ to $1.0x10^2$ CFU/g). Mould count was the highest in South-west ranging from $1.03x10^2$ to $2.331x10^2$CFU/g, while staphylococcal count was high ($2.041x10^2$ to $1.061x10^3$CFU/g). The result indicates that *Masa* contain different type of microorganism like *Shigella spp, Salmonella spp, Pseudomonas sp, E coli* and *Staphylococcus*. Hence Personal and environmental hygiene is required during production of *Masa* to avoid food borne illnesses.

Keywords: *masa*, microbial load, evaluation, process sold, pathogens

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Abbreviations: CFU, colony forming units; g, gramme

Introduction

Masa is a traditional fermented snack usually prepared and consumed in Northern and some part of Western states in Nigeria.[1] It can be produced from maize (Zee mays), millet (*Pennisetumtyphoideum*), sorghum (*sorghum vulgare*) and rice (*Orizasativa*). According to the types of cereals used in preparing *Masa*, we have *Masa* of different types such as; *Masa* Shinkafa, *Masa Masa*ra, *Masa* Gero. *Masa* Dawa. Good quality *Masa* is round in shape with brown colour and smooth surfaces. *Masa* is a Spanish word mostly referred to all kind of dough.[2,3] It is also a popular food among Hausa and Fulani tribes of Nigeria.[4]

The consumption of *Masa* brings about some certain health benefit to consumers because it contained high calories and calcium minerals. It is also a good source of energy and fibre which helps to control appetite and prevent constipation.[5]

Cereal grains are fruit of cultivated grasses of the monocoteledoneus family.[6] Its demand for food makes it abundant in East Asia and Sub-Sahara West Africa. Many researchers have carried out studies on fermentation of cereal product. It is noted that *Masa* is one of the major food that is mostly consumed by student because it is one of the common ready-to-eat food. It is also a fast food with low cost compared to other foods.

It was reported that unhygienic practices among the local food processor usually brings about the contamination of food by microorganisms. This may pose significant health effects to the consumers. In Maiduguri, the outbreak of food borne diseases due to the use of contaminated water has caused the death of many children.[7] In this region of the country, cause of clean water has been advocated. Since it was known that the use of clean water for food preparation influences the outcome on the final products. This includes shelf-life stability, acceptability in terms of sensory qualities and products which pose no health hazards to the consumers. Similarly, the carelessness of the local food vendors, have also been reported.[8] Carelessness in food practices such as the use of unclean utensils and equipment; raw materials selection; selling of foods in open places are potential treats and so increase risk of public health.

Therefore, there was a need to investigate the microbiological quality of *Masa* that is being sold within the University of Maiduguri. This also ensured and ascertained the type of foods produced and sold within the University community.

The method of preparing and selling of *Masa* in an open space in schools may cause contamination to foods. This contamination can also occur due to the use of unclean hands. Water, equipment and utensils during preparation. So there was need to know the types of microorganisms associated with the *Masa* that is being consumed by the students within the university premises to ascertain its safety.

Materials and method

Study area

Handling of foods by traditional food processors and hawkers who do not adhere to strict hygiene practices could pose a threat to public health. There is the risk of foods contaminated with pathogenic microorganisms which could lead to outbreak of food borne illnesses such as staphylococcal food poisoning, shigellosis, salmonellosis and other microbial foodborne illnesses and spoilage microorganisms. Therefore, it became necessary to sample foods from processors and hawkers for microbiological assessment. It was against this programme that this study enumerated, isolated and identified of microorganisms of significance from *masa*, a common convenient food processed and hawked within university of Maiduguri *masa*.

The study area where food samples (*Masa*) were collected is within different locations such as commercial area, hostel area complex area with much population because of the high demand by the student and staff quarters in the university of Maiduguri campus, Borno state.

Description of food

Masa (Figure 1) is a fermented bread-like product, which is round in shape with brown colour and smooth surface made from Millet, maize and rice floor. It is like the "india idle" in shape used in tortilla preparation it is also consumed by all age group in Northern Nigeria, with population of about 47million.[9]

Figure 1 Pictures of *Masa* sampled from various locations within University of Maiduguri campus

Samples and sampling

A total number of 12 *Masa* samples were collected from vendors, 3 from each of the four cardinal point of the university of Maiduguri campus. The samples were collected in sterile containers and labelled. It was taken immediately to the laboratory for analysis.

Microbiological analysis

Sterilization of materials

All the glass wares and materials that were used for the analysis in the laboratory were washed and sterilized in a hot-air-oven at 1600C for one hour.[10]

Preparation of culture media

Culture media were prepared in accordance with the methods described by Collins and Lyne (1970), Harrigan and McCance (1976) Nkama, Badau, Chesbrough, et al.[11–15]

The culture media prepared were Nutrient (NA), Eosin methylene blue (EMB), Mannital salt agar (MSA), Macconkey agar (MA). potato Dextrose agar(POA) and corn meal agar. The media were prepared according to the methods described by Chesbrough.[15]

Preparation of nutrient agar (NA)

Nutrient agar (NA) was prepared by weighing fifty-two grams of the power and dispensing into 1liter of sterile distilled water in a conical flask and was allowed to dissolve completely by swirling. It was sterilized by autoclaving at 12I°C for 5 minutes at 15pounds pressure. It was allowed to cool to a temperature of 45°C and poured into sterile peptic dishes and allowed to gel (solidity). The surface was dried in hot-air-oven before inoculation.[15]

Preparation of eosine methylene blue agar (EMB)

Eosine methylene blue (EMB) was prepared by weighing thirty six grams of EMB powder (Himedia laboratory) into Ilitre of distilled water in a clean control flask and was heated to dissolve completely. It was then sterilized by autoclaving at 121°C at 15psi pressure for 15 minutes. The culture medium was allowed to cool to 45°C before pouring into petri-dishes.[15]

Preparation of mannitol salt agar (MSA)

Mannitol salt agar (MSA) was prepared by dissolving 11grams in 1liter of distilled water, followed by gentle heating to dissolves the medium completely and sterilized by autoclaving at 12I°C, 15 minutes.[15]

Preparation of macconkey agra (MA)

MacConkey agar (MA) was prepared by weighing fifty-two grams of the powder into sterilized flask containig I liter of sterile distilled water. It was sterilized by autoclaving at 121°C, 15psi pressure for 15 minutes. Thereafter mixed well before pouring into petri-dishes and allowed to gel (solidity). The surface was dried in hot-air-oven before inoculation.[15]

Preparation of potato dextrose agar (PDA)

Potato Dextrose agar (PDA) was prepared by dissolving 39grams of media into sterilized conical flask containing 1litre of distilled water and heated to dissolve the medium completely. It was sterilized by autoclaving at 12I°C 15psi pressure for 15minutes.[15]

Preparation of corn meal agar (CMA)

Corn meal agar was prepared by dissolving 17grams of the power into 1litre of sterile distilled water in conical flask and gently heated to dissolve the medium completely, then sterilized by autoclaving at 12I°C 15psi pressure for 15 minutes. This was allowed to cool 45°C before pouring into petri-dishes and allowed to solidity befor use.[15]

Microbiological analysis

Serial dilution

One gram of each sample of *Masa* was weighed and dispensed into 9ml of sterile distilled water as a diluent. 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.[4]

Plating, culture incubation

A pour plate technique was used for plating the *Masa* culture. A triplicate of sterile petri- dishes were labelled corresponding to the number of tube containing the culture. Separate sterile pipettes was used to transfer 1ml of sample from the dilution tube to the corresponding petri-dishes before pouring molten nutrient agar that has been cooled to 45°C after sterilization. This was swirled gently to mix the agar and allowed to solidify before it was incubated at 37°C for 24hours.[15]

Viable cell count

The total plate count for the growth of bacterial colonies on plates was observed by 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/ml) as described by Harriggan and McCance.[16]

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Identification of bacteria

Gram staining techniques: A smear of the specimen was made on slide with a clean glass rod. The film was air dried by waving it over a bunsen flame. The slide was placed on a rack over a sink, cover, smear with crystal violet (the Preioxital stains) reagent and allowed to remain for one minute. The stain was washed off by holding the slide at an angle downwards, and then flooded with iodine solution acting as a mordant. The slide was placed back on the rack and cover with fresh Lugols iodine solution; allowed to react for 1minute. The lugols iodine was pour off and rinsed with gentle running tap water. The slide was also hold in a tilled position and pour acetone reagent slowly until no more dye runs out of the smear to decolourise it. The slide was thoroughly washed with water under gentle running tap water. The whole slides were covered with a counter stain neutral red and allow to reaact for 1 minute. The slide was rinsed slowly under running tap water and blotted with filter paper and allowed to dry in air. A drop of oil immersions was placed in the stained smear and examined using the immersion lens (x100 objective) of a microscope.

Isolation and identification of microorganisms: Microorganisms were isolated and identified based on cultural and morphological characteristics using method described by Harrigan et al.[16] Biochemical tests such as catalase test, coagulase test, indole test, methyl red (MR) test, Voges-Proskauer test, citrate utilization test, urease test and oxidase test were carried out as described by Harrigan & McCance,[16] and Cheesbrough.[15] Results obtained from these tests were compared with literature standards using diagnostic tables showing the biochemical reactions identifying many genera and species of bacteria.[17]

Data analysis

The data obtained were analysed to find the mean using Microsoft word excel (2010).

Results and discussion

The result of microbial load of *Masa* obtained from four cardinal points within university of Maiduguri campus is shown in Table 1. The total bacterial count ranged from $4.26x10^2$ to $6.39x10^2$, $6.80x10^2$ to $7.46x10^2$, $4.27x10^2$ to $5.6x10^2$ and $5.16x10^2$ to $8.33x10^2$ CFU/g of *masa* from North West, North East, South West and South east of University of Maiduguri campus, respectively. Coliform counts of *masa* from North West ranged from $2.40x10^2$ to $2.77x10^2$ CFU/g, North East *masa* had coliform count of $1.33x10^2$ to $2.66x10^2$ CFU/g. Coliform counts of *masa* collected from South West and South East ranged from $1.71x10^2$ to $2.35x10^2$ and $1.39x10^2$ to $3.41x10^2$CFU/g, respectively.

The mould count of *masa* obtained from North West ranged from $1.10x10^2$ to $2.18x10^2$CFU/g, North East *masa* ranged from $1.16x10^2$ to $2.04x10^2$CFU/g, South West had mould count of $1.20x10^2$ to $2.33x10^2$CFU/g and South East *masa* had $1.15x10^2$ to $2.17x10^2$ CFU/g of mould count. The yeast count of *masa* obtained from North West, North East, South West and South East ranged from $1.01x10^2$ to $3.02x10^2$, $1.15x10^2$ to $3.21x10^2$, $1.06x10^2$ to $2.12x10^2$ and $1.04x10^2$ to $3.01x10^2$, respectively. Whreeas, staphylococcal count of *masa* from North West of university of Maiduguri campus ranged from $1.10x10^2$ to $2.01x10^2$, North East, ranged from $1.08\ x10^2$ to $2.04x10^2$, South West, ranged from $1.15x10^2$ to $2.04x10^2$ and South East, ranged from $1.06x10^2$ to $1.18x10^2$ CFU/g.

Table 1 Mean value of microbial load of *Masa* samples from different locations from university of Maiduguri (expressed as cfu/g)

Location	Total bacteria	Coliforms	Coliforms	Yeast	*staphylococcus*
	Count	Count	Count	Count	Count
North West					
NWU1	4.26×10^2	2.27 × 102	1.03×10^2	1.52×10^2	1.10×10^2
NWU2	4.86×10^2	2.77 × 102	2.18×10^2	1.01×10^2	2.01×10^2
NWU3	6.39×10^2	2.40 × 102	1.10×10^2	3.02×10^2	1.11×10^2
North East					
NEU1	6.80×10^2	2.66 × 102	1.30×10^2	3.21×10^2	1.08×10^2
NEU2	7.46×10^2	1.33 × 102	1.16×10^2	1.15×10^2	1.12×10^2
NEU3	6.80×10^2	2.10 × 102	2.04×10^2	2.13×10^2	2.04×10^2
South West					
SWU1	4.27×10^2	1.71 × 102	2.14×10^2	1.08×10^2	2.03×10^2
SWU2	4.53×10^2	2.35 × 102	1.20×10^2	1.06×10^2	1.15×10^2
SWU3	5.60×10^2	2.10 × 102	2.33×10^2	2.12×10^2	2.04×10^2
South East					
SEU1	7.20×10^2	3.41 × 102	1.15×10^2	1.20×10^2	1.12×10^2
SEU2	8.33×10^2	1.52 × 102	2.17×10^2	3.01×10^2	1.06×10^2
SEU3	5.16×10^2	1.39 × 102	2.03×10^2	1.04×10^2	1.18×10^2

KEY: NWU, North west university; NEU, North east university; SWU, South west university; SEU, South east university

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Table 1 above presents the results of the mean value of microorganisms of *Masa* sample from different locations in the University of Maiduguri. The result revealed the total bacterial count from south east of $8.33x10^2$ had the highest bacterial load count while Northwest had the lowest bacterial count of $4.26x10^2$. Colifonns count had the highest count of $3.14x10^2$ from south-east and the lowest count at $1.33x10^2$ from North-east. Yeast count which is $3.2\ Ix10^2$ had the highest count. While $1.01x10^2$ from north- west had the lowest count. Mould count $2.33x10^2$ had the highest count from south west. Staphylococcus count from north-east with $2.04x10^2$ had the highest count while south-east had the lowest count of $1.06x10^2$.

The result obtained from the analysis showed that bacterial count from south-east had the highest count compared to others, which could be as a result of poor processing method, poor hygiene practice, improper and unhygienic handling of the product, bad sanitation operations and use of unclean utensils. This agrees with the fact that immense microbial contamination of food is linked to poor post processing handling practices[18] and the *Masa* from North-west had the lowest bacterial count and it could be as a result of the differences in processing practices.

The presence of coliforms from south-east is high compared to other locations and it can be as a result of unhygienic practices related to feacal contamination, the great increase in the count could be as the location of the retail outlet in which basically heavy contamination occurs. This finding suggests food was exposed to feacal or sewage contamination through the use of contaminated water or contamination from the unsanitary environment, equipment and carriage.[4]

Yeast count is indicated to be the highest from North-east due to direct exposure to air, product is opened as often as the customer's demand and also the handling processes by the vendors,[13] started that the dusty unhygienic environment coupled with the poor handling by vendors are factors contributing to the high microbial load. *Masa* from North-west is lower in yeast count and it could be due to environmental factors.

Mould count is high from south-west which could be as a result of the environmental factors due to dust and open air; therefore, enviromental contaminants have also been implicated as food borne pathogens.[1] North-west had the lowest count which indicates that the environment where the *Masa* is processed is safer.

Staphylococcus had the highest count from North-east which could be as a result of contaminants from personnel through dirty hands, cloths and utensils that comes in contact with the food. Staphvlococcus is common organisms found in all individuals and expelled from the respiratory tract, nose, mouth, clothing, hand and skin.[1]

From the result obtain in Table 2 salmonlla is known as a pathogen that causes typhoid, fever and food poisoning.[19] *Pseudomonas sp* is an opportunistic pathogen that cause bacteremia and gastrointestinal infections[20] *shigella* is a pathgen and its presence has been contributed to unhygienic practices related to faecal contamination.[21,22] *E coli* is an indicator of postprocessing contamination. Staphylococcus is common organisms found in all individuals and expelled from the respiratory track, nose, mouth, clothing, hand and skin. Aboloma have reported that the incidence of staphylococcus in food is an indication of environment and human contamination. It is a pathogen of public health importance.

The percentage occurenrance of microorganisms for "*Masa*" is shown in Table 3. *Salmonella, Shigella* and *Rhizopus Oryza* had 37.5% occurrence in "*Masa*" but the occurrence of *S. aureus, E coli, Pseudomonas, Candida albicas, Saccharomyce cerevisie,* is 25% in *Masa*. The occurrence of all this microorganism is a result of contamination from air could be as a result of unhygienic practices and poor sanitation in and around the environment.[23–25] The dusty, unhygienic environment coupled with the poor handling by vendors are factors contributing to the high microbial load.

Table 2 Morphological and biochemical identification of microbial isolate from "*Masa*"

S/N	Morphological characteristics	Gram reaction	Motility	Co	Ca	Me	In	Ur	Cit	ox	Vp	Organisms isolate
1	Smooth cream opague colonies with entire edges	+Cocci	-	+	+	+	-	+	-	-	-	*Staphylococcus*
2	Smooth pink circular colonies that ferment lactose	-rod	+	-	+	-	+	-	-	-	-	*F. coli*
3	Large grey white mucoid colonies	-rod	-	+	+	-	-	-	+	+	-	*Pseudomonas Spp*
4	Large flat spreading colonies with distint smell	-rod	+	-	+	+	-	-	+	+	+	*Salmonella Spp*
5	Pele colour non lactose fermenting colonies	-rod	-	+	+	+	-	-	+	-	-	*Shigella*

Key: Co, Cogulatase; Ca, Catalase; Me, Methyl red; In, Indole test; Ur, Urease; Cit, Citrate; Ox, Oxidase; Vi, Voges-proskauer; (+), 90% or more positive; No reaction

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Table 3 The percentage occurenrance of microorganisms for "*Masa*"

S/N	Organisms isolate	Sample location				
		N.W	N.E	S.W	S.E	% Occuence
1	*S.aureus*	-	+	-	+	25
2	*Salomonella*	+	+	+	-	37.5
3	*E.coli*	+	-	-	+	25
4	*Shigellia*	+	+	+	-	37.5
5	*Pseudomona*	-	+	-	+	25
6	*Candida albicas*	-	+	+	-	25
7	*Saccharomyce cerevisie*	+	-	-	+	25
8	*Rhizopusoryza*	-	+	+	+	37.5

Keys: (+), Present; (-), absent; N.W, North-west; NE, North-east; SW, South-west; S.E, South-east

Conclusion

The analysis of *Masa* for the isolation and identification of microorganisms has shown that there is higher amount of microbial contaminants in *Masa* due to the way of distribution, use of contaminated water, dirty utensils, the method of service and display in an open air which may be potentially pathogenic to human being and could be a health safety problem.

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Conflict of interest

Author declares that there is no conflict of interest.

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