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Research Paper

Microbiological Changes of Extruded Snacks Made From Orange Fleshed Sweet Potato, Cassava, Plantain, Fortified With *Moringa Oleifera* Powder

Uzoaga. L. N.,and N.A Kanu[2]
1Department of Food Science and Technology, Abia State University. P. M. B. 7010, Umuahia,Abia State, Nigeria.
2 National Root Crops Research Institute Umudike Abia State Nigeria P.M.B 7006
**Corresponding author: Uzoaga. L. N*

Abstract
Aim*: The aim of the study was to assess the shelf life storage of these extruded-on interval of six months. The extruded snacks were developed using locally fabricated extruder from flour of different blend ratio of orange fleshed sweet potato, yellow root cassava, plantain fortified with moringa leaves powder. The microbial quality of extruded snacks was investigated in six months. Eight samples (with different blends) were analyzed for microbial counts and for organisms of public health importance.*
Introduction:
Snacks or ready to eat foods (RTFs) sold on streets have been implicated in foodborne illnesses and its attendant economic loses
Methodology:
Pounds of unripe (green) plantain (Musa paradisiaca normalis) was purchased from a local market in Umuahia, Abia State. Yellow root cassava (Manihot Esculenta Cranz) tuber variety UMUCASS 45 and Orange-fleshed sweet potatoes (OFSP) (Ipomoea batatas .L. Lam) was obtained from the experimental/ commercial farm of the National Root Crop Research Institute (NRCRI) Umudike. Moringa oleifera leaves was obtained from Micheal Okpara University of Agriculture, Umudike (MOUAU) which was sprayed on a tray to dry under natural air (not sundrying). The dried moringa was milled into powder, sieved and packaged in polyethylene bag, sealed and store at room temperature (30 ± 1^oC) until used. Yellow root cassava, Orange-fleshed sweet potato and Plantain were processed into flour. The flour were used to form a blend of eight sample (I= 65% YRC, 20% plantain, 10% OFSP, 5% moringa leaves powder. J = 70% YRC, 15% plantain, 10% OFSP, 5% moringa leaves powder, K= 75% YRC, 10% plantain, 10% OFSP, 5% moringa leaves powder, L= 80% YRC, 10% plantain, 5% OFSP, 5% moringa leaves powder, M= 85% YRC, 5% plantain, 5% OFSP, 5% moringa leaves powder, N= 95% YRC, 5% moringa leaves powder, O= 95% plantain, 5% moringa leaves powder, P= 95% OFSP, 5% moringa leaves powder. The samples were later processed into snacks.The microbial quality of extruded snackswas investigated in six months.
Result:
The total heterotrophic bacterial load among the samples after two months of storage were $5.53\log_{10}$cfu/g ($3.4x10^5$cfu/g); $5.41\log_{10}$cfu/g ($2.6x10^5$cfu/g) $5.59\log_{10}$cfu/g ($3.9x10^5$cfu/g), and 5.Yeast and mould count of the extruded and baked samples obtained in the J category ranged from $2.0x10^3$cfu/g to $1.2x10^4$cfu/g during the six months storage period; while the count recorded for samples K and M ranged between $2.0x10^3$cfu/g to $7.0x10^3$cfu/g and $2.0x10^3$cfu/g to $2.1x10^4$cfu/g respectively during the same period. Generally as expected there was an increase in total heterotrophic bacteria load of all samples with storage time. Moringa leaf aqueous extracts are good prospects for food application because they can preserve food by controlling a wide range of pathogenic microorganisms, such as bacteria and fungi, thereby increasing their shelf life.
Key word: *baked extruded snacks, moringa leaf, plantain, cassava, microbial load*

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I. INTRODUCTION

Snacks are light food eaten in between the main meal to assuage hunger. They are usually used for pleasure rather than the nutrient attached to them Akubor 2004. Currently in Nigeria the demand in snacks is on the increase. They are mainly produced by wheat flour. Wheat flour, the main ingredient for production of snacks are imported and thus, the cost of importation of wheat flour eat deep into the Nigeria economy and has placed a considerable burden on the foreign exchange reserve, in the long run causes increase in wheat products Nwafor *et al*., 2020. The use of locally available flour can be an alternative for the production of snacks. There may be some changes and decrease in quality of snacks during storage due to some factors such asmicroorganism, fat content, moisture, temperature and light, and lipid oxidation, with high moisture contents negatively affecting the flavor, odor, and texture of the productCortez Netto *et al*., 2020.

According to Kanu and Kingsley 2020 incorporation of m*oringa oleifera* seed meal to yam flour improved the nutritional and the phytochemical composition of the blend. Phytochemicals are plant secondary metabolite which possess antimicrobial properties. The extract of the different morphological parts of *moringa* such as the leaf, seeds and pod roots has shown antimicrobial activity against Gram negative and gram positive microorganisms. The seed extract possesses antimicrobial properties against bacteria and fungi species. The antimicrobial properties are as a result of the cationic protein which is known as *moringa oleifera* cationic protein in *moringa*Kanu *et al., 2018.*The research work is aimed at evaluation the microbial changesof extruded snacks made from orange fleshed sweet potato, cassava, plantain, fortified with *moringa* powder

II. MATERIAL AND METHODS

Orange fleshed sweet potato (OFSPI) and yellow root cassava (*Manihot esculenta Crantz)* variety TMS/07/0593 and *Moringa oleifera* leaves was collected from National Root Crop Research Institute Umudike and Plantain (*Musa paradisiaca normalis*) was purchased from Umuahia main market, processed into flour at NRCRI food laboratory. Ingredients like mixed spices, salt and butter were purchased from Umuahia local market. The matured and sorted OFSP tubers, YRC, Plantain were cleaned and washed with tap water to remove any adhering soil, dirt and dust. The tubers were then peeled by with knife. YRC and OFSP were chipped and plantain was sliced to 2 mm thickness with knife to facilitate drying and milling. The sliced plantain and chipped YRC and OFSP were oven dried. The material was then dried in solar drier (Alvant blanch, Withshier, England) for 24 h at 50-60°C till it reached 12% moisture and grounded by miller (Thomas-Wiley laboratory mill, ILCA 5789, Philadelphya PA, USA) with 500 µm screen. The flours were used to form blends at different concentration to obtain eight samples (I= 65% YRC, 20% plantain, 10% OFSP, 5% *moringa* leaves powder. J = 70% YRC, 15% plantain, 10% OFSP, 5% *moringa* leaves powder, K= 75% YRC, 10% plantain, 10% OFSP, 5% *moringa* leaves powder, L= 80% YRC, 10% plantain, 5% OFSP, 5% *moringa* leaves powder, M= 85% YRC, 5% plantain, 5% OFSP, 5% *moringa* leaves powder, N= 95% YRC, 5% *moringa* leaves powder, O= 95% plantain, 5% *moringa* leaves powder, P= 95% OFSP, 5% *moringa* leaves powder). The flour sample was packed in airtight black high-density polyethylene plastic using impulse sealer (HM3000 Polythene heat sealer, hulmemartin, UK) and kept at 15- 18°C until use (these samples were not stored too long and the use of the samples was immediate after the day of preparation).

Preparation of Extruded Snacks Made from a Blend of Yellow Root Cassava Flour, Orange Fleshed Sweet Potato Flour, Plantain Flour and *Moringa oleifera* Leaves Flour

The method described by Yiu(2006) was used for the production of puffed snacks with a little modification. For the production of extruded baked snacks made from blends of yellow root cassava flour, orange fleshed sweet potato flour, unripe plantain flour with m*oringa* leaves powder (used as a fortificant), the dough for the extruded blends was first prepared by mixing 100 g of cassava flour, orange fleshed sweet potato flour, unripe plantain flour and *moringa* leaves powder, 1 g of salt and 1 g of mixed spices, with 150 ml of water in a bowl. The composite flour was thoroughly mixed to the consistency to obtain malleable dough at temperature of 37°C. The dough formed into cylindrical rolls of 5cm in diameter, after which the cylindrical dough was filled in an extruder which was piped on a greased tray. The greased tray with the extruded product was baked in a hot oven (Gallenkamp Co. Ltd. London, England) at 100°C for 30 minutes to the required moisture content (12%). The baked snacks were allowed to cool on kitchen paper and stored in air tight plastic containers prior to various analyses.

The microbial load of the samples were investigated for two mouths storage period. Microbial load was analyzed using Mac-Conkey agar (MA), Deoxycholate citrate agar (DCA), Nutrient agar (NA), and Mannitol salt agar (MSA) for bacteria isolation while Potato Dextrose agar (PDA) was used to isolate the fungi in the microbiology laboratory.

Flour blends of yellow root cassava (YRC), Orange-fleshed sweet potato (OFSP), and Plantain and *Moringa oleifera.*

YRC%	OFSP%	Plantain%	*Moringa* leave powder%
65	10	20	5
70	10	15	5
75	10	10	5
80	5	10	5
85	5	5	5
95	-	-	5
-	95	-	5
-	-	95	5
FLESH ROOT			
100	-	-	-
-	100	-	-
-	-	100	-

YRC- Yellow root cassava, OFSP – Orange-fleshed sweet potato

OFSP- Orange fleshed sweet potato

Microbiological Analysis

Media preparation

The different medium (Nutrient Agar, MacConkey agar, Potato Dextrose Agar and Mannitol Salt Agar) were prepared by weighing out appropriate gram of each agar and dissolving in its equivalent volume of distilled water as stipulated by the manufacturer of each medium. The different mediums were then sterilized in an autoclave for 15mins at 121^0C.

Inoculation of Samples

2.5g of the respective samples were added to a conical flask containing 22.5ml of sterile nutrient broth. The sample was shaken vigorously to homogenize, allowed to stand for 30mins before dilution. Test tubes containing 9ml of sterile distilled water were set up in a rack and labelled 10^{-1} to 10^{-5}. The ten-fold serial dilution techniques was employed in diluting the samples by pipetting 1ml from the conical flask containing the sample into the tube labelled 10^{-1} and properly mixed. 1ml was similarly pipette from this tube to the second tube labelled 10^{-2}. This was repeated for all the tubes till the 5th tube where 1ml was discarded.

After the dilutions, standard microbiological technique was used in spreading each inoculum into several freshly prepared Nutrient Agar (NA), MacConkey Agar, Potato Dextrose Agar (PDA) and Mannitol Salt Agar. 0.1ml was taken from the 4th and 5th dilutions and transferred into the centre of separate NA, and PDA plates. A bent glass rod was used to spread out each inoculum. Glass rod was sterilized by first dipping in ethanol and then passing it quickly through a flame. Each petri dish was properly labelled and incubated at 37^0C for NA plates and at room temperature for the PDA plates. Colony count was performed after 24 hours for bacteria and 48 hours for fungi.

The total bacterial/fungal count expressed in cfu/g was calculated as follows:

TVC = Reciprocal of dilution factor (DF) x number of colonies counted on plate.

Dilution factor (DF) = initial dilution x subsequent dilution x volume of inoculation.

Cfu/g = colony forming unit/gram

TVC = Total viable count

Identification/Characterization of Isolates

Characterization of isolates involves the use of several tests which could either be positive or negative which helps to show the shapes and arrangement of microorganisms. These include:

Gram's staining

The pure isolate was stained as described by Gurung *et al.* (2009). A thin smear of the isolate was made on a clean glass slide and heat-fixed by flaming. Two drops of crystal violet were added to the smear for 1 minute. The drops of crystal was washed with water and stained with Gram's iodine solution for 1 minute. The stain was decolorized by flooding the slide with alcohol until no more violet coloration was observed. Two drops of Safranin reagent was added for 10 seconds rinsed again with tap water and blotted dry using a filter paper. Observation was made using oil immersion objective. Gram-positive bacteria were characterized by purple coloration while gram-negative cells were pinkish in colour. This staining technique also shows the different shapes and arrangement of the bacteria cells.

Biochemical Test

Catalase Test

2-3 drops of 3% hydrogen peroxide was placed on a clean grease-free slide using a pipette and a loopful of pure culture of the isolates was added and emulsified. Formation of visible bubbles was taken as a positive test while absence of bubbles was regarded as a negative test (Cheesbrough, 2006).

Methyl Red Test

The isolates were inoculated into various MR-VP (Methyl Red Voges- Proskauer) broth bottles and appropriately labeled. The bottles will be incubated for five days. After incubation, five drops of methyl red were added to the bottles for methyl red test. A positive test was indicated by appearance of red color. Negative test was indicated by a yellow coloration of the media (Cheesbrough, 2006).

Voges- Proskauer (VP) Test

The isolates were inoculated into various MR-VP broth bottles and appropriately labeled. The bottles were incubated for five days. After incubation, 1ml of the MR-VP broth with test organisms was transferred to sterile test tubes. 15 drops of VP reagent A was added to the test tubes followed by 5 drops of VP reagent B. A positive test was indicated by appearance of pink-red color. Negative test is indicated by a light yellow coloration of the media (Cheesbrough, 2006).

Indole Test

The isolates were inoculated into a bijou bottle containing 3ml of sterile Tryptone broth and incubated at 35-37°C for 48 hours. Indole production was tested by adding 7 drops of Kovac’s reagent and examined for formation of a ring of red color in the surface layer within 5 minutes. A ring of red layer indicates a positive test. Absence of a ring of red layer indicates a negative test (Cheesbrough, 2006).

Citrate Utilization Test

Thiswas used to detect the ability of each isolate to use citrate as its sole source of carbon and energy. Slopes of Simmons citrate agar were prepared in bijou bottles following the manufacturer’s directives. Using a sterile straight wire, the slope was first streaked with a saline suspension of the isolates and then the butt stabbed and incubated at 35°C for 48 hours. A bright blue color was recorded as a positive test (Cheesbrough, 2006).

Hydrogen Sulphide (H_2S) Production Test

Hydrogen sulphide (H_2S) production test was used for the detection of hydrogen sulphide (H_2S) gas produced by an organism. An inoculum from each isolate was transferred aseptically to a sterile triple sugar iron agar (TSIA) slant. The inoculated slants were incubated at 35°C for 24hours and the results observed. Hydrogen sulfide combines with the iron in the media to produce iron sulfide (FeS). The presence of H_2S producing organism is detected by the turning of the agar slants to black colour.

Starch Hydrolysis

Equimolar amount of soluble starch was prepared and added to MRS agar without glucose or meat extract to give a 1% soluble starch before being poured to set in sterile plates. Single streaks of cultures were made on the dried plates before being incubated at 30ºC for 48 hours. The plates were flooded with Gram’s iodine after incubation. Unhydrolysed starch formed a blue colouration with iodine. Clear zones around the region of growth indicated starch hydrolysis by the culture (Seely and Vann demark 1972).

Fungal Identification

Fungal isolates was identified macroscopically and microscopically using lactophenol cotton blue in which a portion of the fungi was collected using sterile needle and placed on the microscope slide. A few drops of lactophenol cotton blue will be added on it, followed by teasing using needle. Later they were covered with cover slips and viewed under the microscope using X10 and X40.

III. RESULT AND DISCUSSION

Total Bacteria Load on table 1

Total viable count reflects the conditions in which a food is produced, stored, or abused during handling and can be used to predict the shelf life or keeping quality of the product. The spoilage of many foods may be imminent when the total viable count reaches from 10 –100 million per gram of the product (Nagi *et al.,* 2012). The total microbial load for the samples observed on nutrient agar for bacteria and Potato-dextrose agar for fungi were within the range of 6.0×10^4cfu/g to 2.7×10^5cfu/g and 3.0×10^3cfu/g respectively within two weeks of production (Table 1).

Although, all microorganisms were expected to have been destroyed during baking due to high temperature, during packaging, cross contamination could occur due to handling of the products resulting in the bacterial loads recorded after the two weeks of production.

The total heterotrophic bacterial load among the samples after two months of storage were 5.53log_{10}cfu/g (3.4x10^5cfu/g); 5.41log_{10}cfu/g (2.6x10^5cfu/g) 5.59log_{10}cfu/g (3.9x10^5cfu/g), and 5.51log_{10} cfu/g (3.2x10^5cfu/g) for samples L, M, N and J respectively (Table 1). It has been reported by Wareing *et al.* (2001) that the high perishable nature of cassava tubers makes them susceptible to contamination by bacteria. The counts obtained here are comparable to those of Gacheru *et al.* (2016) in a similar study, where bacterial count of dried cassava chips samples were in the range 2.69-4.36logcfu/g and 2.90-4.71logcfu/g with a mean count of 3.65logcfu/g and 3.79logcfu/g, respectively. The high temperature from the extrusion cooking process could have resulted in the reduced microbial contamination recorded in this investigation. Similar effects of extrusion cooking have been earlier reported in previous studies by Roland and Stanley, (2011).

The bacterial load obtained from samples K and P (4.4x10^5cfu/g each) was the least throughout the 6months of storage when compared with other samples (Table 1). The probable reason for more increased load of microorganisms in the samples L, M, and N which had higher composition of cassava flour could be attributed to the susceptibility of cassava to the microbial attacks. However the results obtained from the other samples revealed moderate level of microbial contamination that could be attributed to the manual and post-processing handling practices.

Total microbial load increased steadily but minimally. The increase in microbial load as the storage period lengthened might have been due to a corresponding increase in moisture contents during storage and availability of nutrient in the samples (Braide *et al.,* 2012). The finding of this investigation is in line with those of earlier studies including those of Ubbor and Akobundu, (2009); Helen and Ndidiamaka, (2014).

The *Moringa oleifera* extract could also have contributed in retarding the growth of microorganisms over time. Generally as expected there was an increase in total heterotrophic bacteria load of all samples with storage time. The increased load of microorganisms as the period of storage could also be ascribed to the loss in potency of the preservative (*Moringa olifera*) with time. Seetaramaiah *et al.* (2011) had earlier reported that the quantitative phytochemical screening of *Moringa oleifera* revealed the presence of flavonoids alkaloids, tannins, saponins and cyanogenic glycosides for bioactive compounds which in correct doses can successfully be used to inhibit and eventually destroy microorganisms. Neall, (2006) reported that *Moringa oleifera* has a broad spectrum antimicrobial activity, which works against most bacteria (Gram-positive and Gram-negative). On the contrary, other reports indicated that inclusion of *Moringa* leaf extracts, at relatively low doses, in meat products did not affect the microbial load of the products (Muthukumar *et al.,* 2014; Shah *et al.,* 2015).

Table 1: Total Heterotrophic Bacterial Counts of Samples during Storage

Table 1: Total Heterotrophic Bacterial Counts of Samples during Storage

Period of storage	Total Viable Count (cfu/g)							
	I	J	K	L	M	N	O	P
April (2weeks)	8.0x10^5	NG	NG	1.2x10^5	6.0x10^5	1.9x10^5	2.7x10^5	1.6x10^5
April (4weeks)	1.2x10^5	4.0x10^5	8.0x10^5	1.4x10^5	6.0x10^5	2.4x10^5	2.8x10^5	1.7x10^5
May (6weeks)	2.6x10^5	7.0x10^5	1.1x10^5	2.9x10^5	1.8x10^5	3.3x10^5	3.1x10^5	2.1x10^5
May (8weeks)	3.1x10^5	1.1x10^5	1.6x10^5	3.4x10^5	2.6x10^5	3.9x10^5	3.4x10^5	2.9x10^5
June	3.8x10^5	3.2x10^5	2.6x10^5	4.6x10^5	4.1x10^5	4.4x10^5	3.9x10^5	3.3x10^5
July	4.3x10^5	3.9x10^5	2.9x10^5	5.3x10^5	4.8x10^5	4.6x10^5	4.1x10^5	3.7x10^5
August	5.8x10^5	5.1x10^5	3.8x10^5	5.8x10^5	6.1x10^5	4.6x10^5	5.2x10^5	4.3x10^5
September	6.3x10^5	5.4x10^5	4.4x10^5	6.6x10^5	7.2x10^5	5.9x10^5	5.4x10^5	4.4x10^5

The total bacterial load was enumerated on Nutrient Agar plates.
The total microbial load was obtained from:
Microbial load = reciprocal of dilution factor x Number of resultant colonies on Petri dish
Where dilution factor (DF) is given by:
DF = Initial dilution used x Subsequent dilution of Inoculation x Volume of inoculation
cfu/g = colony forming unit per gram.

Fungal (Yeast and Mould) count, table 2

Yeast and mould count of the extruded and baked samples obtained in the J category ranged from 2.0x10^3cfu/g to 1.2x10^4cfu/g during the six months storage period; while the count recorded for samples K and M ranged between 2.0x10^3cfu/g to 7.0x10^3cfu/g and 2.0x10^3cfu/g to 2.1x10^4cfu/g respectively during the same period (Table 2). The fungal counts obtained from the samples were considerably low and some of the samples had no growth of fungi at all on two months after production. This may be attributed to drying of the samplesto considerably low moisture content. These results agree with a report by Ogori and Gana (2013), the low counts could have resulted from the low moisture content that limited microbial growth. The results of this study is also comparable to that reported by Kaaya and Eboku (2010) that showed a mean mould count of 7.0x10^3cfu/g. Low moisture content is a very significant parameter in foods as they help to enhance the shelf life of food samples and prevent rapid spoilage by microorganisms (Pupulawaththa *et al.,* 2004). It was however observed that as the duration of storage increased the load of fungi also increased though by a narrow margin.

The fungal load after the third and fourth month for samples L were $8.0x10^3$cfu/g and $1.8x10^4$cfu/g respectively whereas for sample N it was $1.6x10^4$cfu/g and $2.7x10^4$cfu/g respectively after the third and fourth month (Table 2). The little increase observed during storage could be attributed the absorption of moisture by the samples during the period of storage. In a related study by Kaaya and Eboku (2010), their samples were contaminated with *Penicillium*, *Aspergillus* and *Fusarium* species. Fungal contamination can lead to discoloration of the products, give rise to mouldy taste and produce off odours Adebayo-Oyetoro *et al.,* 2003. Growth of molds could result in production of mycotoxins, which are toxic secondary metabolites. According to Williams *et al.* (2004), mycotoxin exposure contributes to more than 40% of the global disease burden.

Table2: Total Fungal Counts of Samples during Storage

The total fungal load was enumerated on Potato Dextrose Agar plates.
The total microbial load was obtained from:

Period of storage	Total fungal count (cfu/g)							
	I	J	K	L	M	N	O	P
April (2weeks)	NG	NG	NG	NG	NG	$3.0x10^3$	NG	NG
April (4weeks)	NG	NG	NG	$3.0x10^3$	NG	$3.0x10^3$	NG	NG
May (6weeks)	$4.0x10^3$	NG	NG	$5.0x10^3$	$2.0x10^3$	$8.0x10^3$	NG	NG
May (8weeks)	$4.0x10^3$	NG	NG	$5.0x10^3$	$5.0x10^3$	$1.1x10^4$	NG	NG
June	$4.0x10^3$	$2.0x10^3$	NG	$8.0x10^3$	$1.1x10^4$	$1.6x10^4$	$4.0x10^3$	$2.0x10^3$
July	$7.0x10^3$	$3.0x10^3$	$2.0x10^3$	$1.2x10^4$	$1.4x10^4$	$2.2x10^4$	$7.0x10^3$	$6.0x10^3$
August	$7.0x10^3$	$7.0x10^3$	$5.0x10^3$	$1.6x10^4$	$1.7x10^4$	$2.5x10^4$	$1.3x10^4$	$8.0x10^3$
September	$1.3x10^4$	$1.2x10^4$	$7.0x10^3$	$1.8x10^4$	$2.1x10^4$	$2.7x10^4$	$1.7x10^4$	$8.0x10^3$

Microbial load = reciprocal of dilution factor x Number of resultant colonies on Petri dish
Where dilution factor (DF) is given by:
DF = Initial dilution used x Subsequent dilution of Inoculation x Volume of inoculation
cfu/g= colony forming unit per gram.

Staphylococcal Bacteria Count table 3

Total Staphylococcalcount in samples ranged from $8.0x10^2$cfu/g to $4.2x10^3$cfu/g for samples in the M varieties; from $6.0x10^2$cfu/g to $1.9x10^3$cfu/g for N varieties and $2.0x10^2$cfu/g to $2.3x10^3$cfu/g for P varieties (Table 3). Similarly, the counts obtained for samples J and K ranged between $3.0x10^2$cfu/g to $2.0x10^3$cfu/g and $2.0x10^2$cfu/g to $1.8x10^3$cfu/g respectively (Table3). As with both the total heterotrophic bacterial and fungal counts the counts obtained for the staphylococcal species were continually increasing. *Staphylococcus aureus* was isolated from all the varieties. The presence of *Staphylococcus aureus* is usually an indicator of unhygienic production processes like handling and exposure both at the processing sites and in packaging/storage (Obadina *et al.,* 2008). The results in the present study are in agreement with those found by Ogori and Gana (2013).

It is recommended that food processors be trained on good hygienic practices, adoption of best practices in processing, of raw materials prior to drying. *Staphylococcus aureus* are potential pathogens and thus disease causing microorganisms that may enter the body through the ingestion of food, causing food borne illnesses which are a leading cause of illness globally killing an estimated 2.1 million people annually, most of whom are children in the developing world (WHO, 2001). These microorganisms found in these products may be due to poor handling during harvesting, processing (especially where the quality of water used may impact greatly on the type of microorganisms that are present in the products), storage or transportation.

Table3: Total Staphylococcal and Coliform counts of Samples during Storage

Period of storage	Total Staphylococcal Count (cfu/g)								Total Coliform Count (cfu/g)							
	I	J	K	L	M	N	O	P	I	J	K	L	M	N	O	P
April (2wks)	$2.0x10^2$	NG	NG	$6.0x10^2$	$8.0x10^2$	$6.0x10^2$	$3.0x10^2$	NG	NG	NG	NG	NG	NG	NG	NG	NG
April (4wks)	$3.0x10^2$	NG	$2.0x10^2$	$9.0x10^2$	$1.2x10^3$	$6.0x10^2$	$5.0x10^2$	$2.0x10^2$	NG	NG	NG	NG	NG	NG	NG	NG
May (6wks)	$3.0x10^2$	NG	$4.0x10^2$	$1.6x10^3$	$1.5x10^3$	$6.0x10^2$	$5.0x10^2$	$4.0x10^2$	NG	NG	NG	NG	NG	NG	NG	NG
May (8wks)	$4.0x10^2$	$3.0x10^2$	$6.0x10^2$	$1.9x10^3$	$1.5x10^3$	$7.0x10^2$	$5.0x10^2$	$1.3x10^3$	NG	NG	NG	NG	NG	NG	NG	NG
June	$5.0x10^2$	$8.0x10^2$	$1.1x10^3$	$2.6x10^3$	$1.6x10^3$	$1.3x10^3$	$8.0x10^2$	$1.3x10^3$	NG	NG	NG	NG	NG	NG	NG	NG
July	$5.0x10^2$	$1.4x10^3$	$1.4x10^3$	$2.8x10^3$	$2.4x10^3$	$1.5x10^3$	$1.2x10^3$	$1.9x10^3$	NG	NG	NG	NG	NG	NG	NG	NG
August	$1.4x10^3$	$1.7x10^3$	$1.4x10^3$	$4.2x10^3$	$3.1x10^3$	$1.5x10^3$	$1.3x10^3$	$2.2x10^3$	NG	NG	NG	NG	NG	NG	NG	NG
September	$2.3x10^3$	$2.0x10^3$	$1.8x10^3$	$4.6x10^3$	$4.2x10^3$	$1.9x10^3$	$1.6x10^3$	$2.3x10^3$	NG	NG	NG	NG	NG	NG	NG	NG

The total Staphylococcal and Coliform load was enumerated on Mannitol Salt Agar and MacConkey agar plates respectively.
The total microbial load was obtained from:
Microbial load = reciprocal of dilution factor x Number of resultant colonies on Petri dish
Where dilution factor (DF) is given by:
DF = Initial dilution used x Subsequent dilution of Inoculation x Volume of inoculation
cfu/g = colony forming unit per gram.NG= No growth

Coliform CountsTable3

From the present study, it is indicated that all the samples were free from coliform during storage periods. Although the presence of *E. coli* and other coliforms were investigated in this study, it could not be detected in any of the samples. This could be ascribed to good manufacture hygiene. The results of this investigation suggested that selective inactivation and inhibition of Gram-negative bacteria had occurred. Also the results of this study recognized that *M. oleifera* leaves extract had antimicrobial properties which may be used as natural preservative material for the products. This finding is in corroboration with the observation of Salem *et al.* (2013), who reported that they do not detected any yeast and mold and coliform bacteria in Labneh prepared with *M. oleifera* extract.

The coliform count was however not significant as shown in (Table 3). The counts obtained from the samples were considerably low and most of the samples did not harbor the coliforms. The presence of these pathogenic microorganisms in any food product is usually an indication of unhygienic production processes resulting in microbial contamination with an incessant risk to the health of man (Odetunde *et al.,* 2014). Their absence in these products makes them safe for consumption.

The high level of hygiene during processing of cassava, potato and plantain flour could be responsible for minimal variation of microbial population (total heterotrophic count, total fungal count, coliform and Staphylococcal count) among the samples as represented in Table 1, Table 2 and Table 3

This result is in agreement with bacteria genera isolated from plantain flour retailed at Urban market in Ondo state, Nigeria Aruwa and Ogundare, (2017). In another related study, Gacheru *et al.* (2016) reported that *Staphylococcus* sp. and *Escherichia coli* were present in cassava flour sold in Nairobi and coastal regions in Kenya. Omohimi *etal.* (2019) in a related study isolated Staphylococcus sp. and several species of molds in cassava flour. According to International Commission on Microbiological Specification for Food (ICMSF), total bacterial count in food should not exceed 1 x 10^6cfu/g; *Staphylococcus aureus* should not exceed 1 x 10^5cfu/g and total coliforms should not exceed 1 x 10^4cfu/g (Omohimi *et al.,* 2019). Based on ICMSF specification, total heterotrophic count of the respective samples were very close to recommended safe limits. Considering Staphylococcal and total coliform count in the samples, the values obtained were within the range recommended by ICMSF (1998). This study further revealed that the samples had a relatively lower total viable count. This observation is in line with those of Djeri *et al.* (2010) who reported similar findings from a related study.

The identification procedure on table 4 showed that a total of nine (9) microorganism (including five bacteria and three fungi) were isolated from the samples. This consisted of five (5) bacterial isolates which were confirmed as *Bacillus* sp*, Staphylococcus aureus, Enterobacter sp., Streptococcus* sp., *and Micrococcus luteus,* following series of biochemical reactions of the isolates. Out of the samples evaluated throughout the 6months period, nine (9) different microorganism where isolated, with *Staphylococcus aureus* being the most predominant isolate found in almost all the samples during storage. Table 4 shows that the four fungal isolates were confirmed as *Aspergillus niger, Penicillium* sp*., Fusarium solani* and *Rhizopus stolonifer* with reference to Barnett and Hunter (1986). Finally since the presence of pathogenic microorganisms as well as other microbial contaminants is almost impossible to get rid of; it is paramount that basic hygiene and sanitary rules should be observed in the production chain. Capacity building of processors on good practices especially good hygiene practices (GHP) and good storage practices (GSP) as well as equipping them with more affordable options of

hygienic drying equipment and methods, will lead to improved quality and safety of the cassava products available in the market (Olowoyo *et al.,* 2001).

M. oleifera leaves extract had excellent antimicrobial properties which may be used as natural preservative material. *Moringa* leaf aqueous extracts are good prospects for food application because they can preserve food by controlling a wide range of pathogenic microorganisms, such as bacteria and fungi, thereby increasing their shelf life. This study will help food processors to increase the shelf life and quality of different products.

Table 3: Morphological and Biochemical Characteristics of the isolates

Colonial Description	Gram status	Cell shape	Indole rxn	Catalase test	Citrate	H_2S test	Starch hydrolysis	Probable Organism
Creamy coloured colonies with irregular shape on NA plate	+ve	Rods	-ve	-ve	+ve	+ve	-ve	*Bacillus* species
Smooth yellow coloured colonies on MSA plates.	+ve	Cocci in clusters	Na	+ve	na			*Staphylococcus aureus*
Large pink colonies on MCA plates	-ve	Rods	-ve	+ve	+ve	+ve	-ve	*Enterobacter aerogenes*
Tiny Yellow coloured colonies on NA plates	+ve	cocci	-ve	+ve	+ve	-ve	-ve	*Micrococcus* species
Creamy mucoid circular Large flat colonies on NA plates	+ve	Cocci in chain	-ve	-ve	+ve	-ve	-ve	*Streptococcus* species

Table 4: Macroscopic Characteristics of the Fungal Isolates

Macroscopic characteristics	Microscopic features	Probable fungi
Bright green colonies with white edges on PDA plates	Long slender conidiophores branched at the apex with several conidia bearing septate hyphae	*Penicillium* species
Rapidly growing colonies producing cotton-like mycelia that grow to the brim of PDA plates	Upright sporangiophores borne on aseptate hyphae. Sporangia are borne on the sporangiophores with spherical spores	*Rhizopus stolonifer*
Profuse growth of brownish black spreading colonies developing from the centre of the plates	Conidiophores are upright and uniform in thickness. The conidiophores were borne on a septate hyphae	*Aspergillus niger*
White coloured colonies with cottony growth raised mycelium with smooth margin	Long aerial unbranched conidiophores, slightly narrow towards the apex; few oval septate producing conidia.	*Fusarium* species

IV. CONCLUSION:

M. oleifera leaves extract had excellent antimicrobial properties which may be used as natural preservative material. *Moringa* leaf aqueous extracts are good prospects for food application because they can preserve food by controlling a wide range of pathogenic microorganisms, such as bacteria and fungi, thereby increasing their shelf life

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