



Research Paper

## Assesment of Microbial Population of Cooked Cassava (*Manihot esculenta*) From Vendors in Sokoto Central Market

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### ABSTRACT

The Study was carried out to determine the microorganisms associated with cooked cassava products sold in Sokoto Central Market, Sokoto State. Five different samples of cooked cassava were randomly collected from five different vendors at the Market using sterile plastic containers. The samples were taken to the Mycology Laboratory in Department of Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto for microbial analysis. Nutrient Agar (NA) and Potatoes Dextrose Agar (PDA) were the media used for growth of the isolates. The result indicated that twelve bacteria species (*Staphylococcus aureus*, *Lactococcus lactis*, *Bacillus cereus* *Bacillus megaterium*, *Alcaligenes faecalis*, *Citrobacter freundii*, *Bacillus subtilis*, *Aeromonas hydrophila*, and four fungal species (*Aspergillus niger*, *Aspergillus fumigates*, *Aspergillus oryzae* and *Saccharomyces cereviceae*) were isolated and identified from the cooked cassava samples. *Bacillus megaterium* had the highest frequency with 15.4% and leaset were recorded on *Bacillus subtilis* and *Lactococcus lactis* recorded the least with 7.7%. *A. niger* was observed to have the highest frequency of occurrence and the least was *A. fumigatus*. The cooked sold by vendors harbour a microbial population that could pose a health harzard and therefore should be avoided unless good sanitary practice are put in place.

**Keywords:** Cooked, cassava, microorganisms, mycology.

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### I. INTRODUCTION

Cassava (*Manihot esculenta*) is a dicotyledonous plant and widely grown root crop in tropical regions of Africa, Latin America and Asia (Ihenkoronye and Ngoddy, 2005). Cassava is grown throughout the tropics and could be regarded as the most important root crop in terms of area cultivated and total production. Fresh cassava roots cannot be stored for long because they rot within days of harvest since they are bulky with about 75% moisture Content, therefore, it must be processed into various forms in order to increase the shelf-life of the products, facilitate transportation and marketing, reduce cyanide content and improve portability (Emurotu, *et al.*, 2012). Microbiological quality of food indicates the amount of microbial contaminants it has, a high level of contamination indicates how level of food storage and its handling and is more likely to transmit diseases (Monday, *et al.*, 2014). Bacterial count in prepared food is a key factor in assessing the quality and safety of food. Food and water in particular have been described as vehicle for the transmission of microbial diseases (WHO, 2011).

Safe food is a basic human right despite the fact that many foods are frequently contaminated with naturally occurring pathogenic microorganisms which cannot be detected organoleptically (seen, smelled or tested) but can cause, disease (including death especially if the way they are conserved during processing and exposition for sales provides condition for those microorganisms to grow and reach considerable levels of contamination (WHO, 2002). Food borne illness is a major international health problem and an important cause of reduced economic growth (Frenzen, *et al.*, 2005). The problems of food safety in industrialized world differ considerably from those faced by developing countries, traditional methods are used for processing and packaging of fresh produce while in developed countries, high standard is employed (Akinsanya, *et al.*, 2013).

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The global incidence of food borne diseases is difficult to estimate but it has been reported that in 2010 alone about 2.7 million people died from food borne diseases alone (WHO, 2005).

In Nigeria, the processing, sale and distribution and marketing of cassava products such as boiled cassava, abacha, garri and akpu in local markets is associated with unwholesome and unhygienic practices which may lead to microbial contamination due to transfer of microbes from dirty hands and utensils and frequent visits by animals and fomites which increases microbial load of these products. Therefore the study aimed to evaluate the microorganisms by Isolating and identifying bacteria and the fungi associated with cooked Cassava; and to also determine the percentage frequency of occurrences of the microorganisms associated with the cooked cassava. Thus ascertain the level of contamination of the cooked cassava samples and the possibility of posing health problems on the consumer.

## II. MATERIALS AND METHODS

### Collection of Samples

Five (5) cooked samples of Cassava were purchased from five (5) different vendors in Sokoto central market. They were separately packed in polythene bags and transported to Microbiology laboratory Usmanu Danfodiyo University sokoto, for analysis.

### Preparation of Media

All the media used in this study were prepared and sterilized according to manufacturer's instruction. The media used include Nutrient Agar (NA), and Potato dextrose Agar (PDA). Nutrient Agar and Potato dextrose Agar (PDA) powder Are weight using weighing balance and diluted using sterile water and autoclaved at 121°C for 15minutes (Adesemoye and Adedire, 2005).

### Sample Processing

One gram of cooked crushed cassava samples bought from Sokoto central market was added into a beaker containing 10ml of distilled water in a ratio of 1:10 and were mixed thoroughly until the solution homogenized. A ten-fold serial solution was carried out as previously described (Inetianbor *et al.*, 2014). The same procedure will be repeated for other boiled samples obtained from the markets. Bacteria will be grown in nutrient agar at 37°C for 24hrs. Pure cultures of different isolates will be obtained and stored in a nutrient broth slant (Cheesbrough, 2006). fungi inoculant were grown in potato dextrose agar for 3-5days at 37°C at room temperature and identified using their cultural and morphological characteristics (Fawole and Oso, 2004). Cultural and morphological characterizations of the bacteria and fungi isolates were determined accordingly to (Harriga and McCance, 2006).

### Gram Staining:

Using a sterile loop, a light suspension of organism in sterilized water was prepared in a clean microscope slide. The film will be air-dried and heat-fixed by passing the slide twice through a gas flame. The slide will then allow cooling. The slide will be placed on a staining rack flooded with crystal violet solution and left for 30 seconds before washing off with running tap water. The slide was again flooded with Lugol's iodine solution and left for 30 seconds before washing off with running tap water. To decolorize, 50:50% acetonealcohol was run over the film and washed off immediately with distilled water. The film was flooded with safranin solution and left for 1min before washing off with distilled water. A drop of immersion oil was then placed on the film and was examined under the microscope using the x100 oil inversion lens. Dark purple color indicated gram positive reaction and pink color indicated gram negative reaction.

### Biochemical Test

The Biochemical testing for identification of bacterial isolate was carried out by conducting Catalase, Coagulate, Urease, Citrate utilization test, Indole, MR-VP test, Starch and Tripple Sugar Ion tests.

#### Catalase Test

A drop of hydrogen peroxide was placed on a slide and a 24 hours growth culture was emulsified with the drop of hydrogen peroxide on the slide. Immediately it was observed for the present of bubbles as indication for positive reaction, absence of bubbles indicates negative result (Cheesebrough, 2006).

#### Coagulase Test

A drop of the water on a slide and a pure culture is then emulsified with the drop of water on the slide to a suspension. A drop of blood is then mixed on the slide and it was immediately observed for agglutination, a positive test indicates plasma has undergone clotting (Oyeleke and Manga, 2008).

#### Urease Test

Urea agar slants in universal bottles were inoculated (streaking) with 24 hours old culture and inoculated at 37°C for 48hours. Positive test was indicated by pink color as a result of an enzyme urease which hydrolysed the urea to carbon dioxide and ammonia, while negative test was indicated by absence of pink color (Oyeleke and Manga, 2008).

### Citrate Utilization Test

A twenty four hours culture of the isolates was inoculated into slanted test tubes containing sterile Simmons citrate medium aseptically and incubated at 37°C for 72 hours, observing daily for the presence of growth and color changes, positive reaction was indicated by the development of deep blue color. (Oyeleke and Manga, 2008).

### 3.5.5 Indole Test

24 hours old culture was inoculated into a test tube containing sterile nutrient broth and incubated at 37°C for 48 hours. 5 drops of Kovacs' indole reagent was added to the 48 hours broth culture which was then shaken. A positive reaction was indicated by the appearance of red ring coloration on top of the reagent above the broth medium within 1 minute. While in negative reaction, the indole reagent retains its yellow color. (Barrow and Falham, 1993).

### MR-VP Test

Isolates were inoculated into MR-VP medium in test tubes and incubated at 35°C for 48 hours. Two drops of methyl red indicator were added after the period of incubation. Positive test was by red color while negative test showed yellow color (Oyeleke and Manga, 2008). Five drops of potassium hydroxide (KOH) was added to 48 hours old MR-VP medium culture of isolates. 2 to 3 drops of alpha naphthol was added and shaken. The test tube is sloped (slant) and examined for 1 hour. Positive test was indicated by strong red coloration while negative result showed no red color (Oyeleke and Manga, 2008).

### Starch Test

Nutrient agar was prepared and fortified with 0.5% of starch after which the content was then dissolved and sterilized and then poured into sterile plate to solidify. The inoculated organisms incubated at 37°C for 24 hours for growth, after then few mils of iodine were spread over the growth. Color change was observed which indicate blue-black as negative.

### Triple sugar ion Agar

Triple sugar ion agar slants in test tubes were cultured with isolates (24 hours old culture) using a sterile needle. The surface was streaked and butt was stabbed 3 times. The test tubes were incubated at 37°C for 24 hours after which they are examined for gas production, glucose, sucrose, and lactose fermentation including motility and hydrogen sulphide respectively. For a positive reaction, glucose fermentation is indicated by butt becoming yellow, while in lactose and sucrose, the media appeared yellow. Gas production was determined by the appearance of bubbles or crack in the media. Motility was positive due to cloudy medium and line of inoculation not sharply defined, a black color along the line of stabbing indicate a positive reaction for hydrogen sulphide (Oyeleke and Manga, 2008).

### Isolation procedure of fungi from cooked cassava

Three sterile conical flasks each containing 100ml of distilled water were set and 1g of each three different samples were immersed into each flask and shaken as its required. A set of 9 test tubes each containing 9ml of distilled water were arranged (3 test tubes for each sample) for serial dilution. From each test tube 0.1ml was taken into a sterile petri-dish containing PDA media using pipette and was gently spread with bend glass for spread plate method (Anupama, 2021). The plates were incubated at room temperature for 3 to 5 days. After incubation the fungi were sub cultured to get a pure isolate.

### Identification of isolated fungi from cooked cassava

The sub cultured isolate were subjected to microscopic examination with a view to identify the associated fungi and is limited to generic level. A new glass slide was sterilized with cotton wool soaked with alcohol. Inoculating needle was sterilized and a drop of Lacto-phenol cotton blue was placed at the center of the slide. A portion of fungal mycelium was removed using inoculating needle and placed on a clean microscopic slide. The slide was then mounted under microscope using X40 objective lens and examined (Shovon, 2015).

## III. RESULTS

The result obtained in this study was presented in tables. The result biological test showed the biochemical test results of the bacteria isolated which include *Lactococcus lactis*, *staphylococcus aureus*, *Bacillus megaterium*, *Bacillus cereus*, *Alcaligenes faecalis*, *Bacillus subtilis*, *citrobacter freundii*, and *Aeromonas hydrophilia* Table 1.

GRAM REACTION	GLUCOSE	LACTOSE	SUCROSE	MOTILITY	GAS	CITRATE	INDOLE	MR	H <sub>2</sub> S	VP	UREASE	CATALASE	STARCH	COAGULASE	SPECIES
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POSITIVE COCCI	+	+	+	-	-	-	-	-	-	+	-	-	-	NA	<i>Lactococcus lactis</i>
POSITIVE COCCI	+	+	+	-	+	+	-	-	-	+	-	+	-	+	<i>Staphylococcus aureus</i>
POSITIVE ROD	+	-	+	+	-	+	-	+	-	-	+	+	+	NA	<i>Bacillus megaterium</i>
POSITIVE ROD	+	-	+	+	-	+	-	-	-	+	-	+	+	NA	<i>Bacillus cereus</i>
NEGATIVE ROD	-	-	-	+	-	+	-	+	-	-	-	+	-	NA	<i>Alcaligenes faecalis</i>
NEGATIVE ROD	+	+	+	+	+	+	-	+	+	+	-	-	-	NA	<i>Citrobacter freundii</i>
POSITIVE ROD	+	-	+	+	-	+	-	+	-	-	+	+	+	NA	<i>Bacillus subtilis</i>
NEGATIVE ROD	+	+	+	+	-	+	+	+	+	-	-	+	+	NA	<i>Aeromonas hydrophila</i>

**KEYS:** SI=Isolate Code, MR=Methyl Red, VP=Voges Proskauer, H2S= Hydrogen sulphide

The result in **Table 2** showed the Percentage Frequency of occurrences of bacteria isolate from cooked cassava market samples. The frequency of occurrences indicated that *Lactococcus lactis* (7.7%), *staphylococcus aureus* (15.4%), *Bacillus megaterium* (23%), *Bacillus cereus* (15.4%), *Alcaligenes faecalis* (7.7%), *Bacillus subtilis* (7.7%), *citrobacter freundii* (15.4%), *Aeromonas hydrophilia* (7.7%).

**Table 2:** Percentage frequency of occurrences of bacteria isolates.

BACTERIA IDENTIFIED	NUMBER OF OCCURRENCE	PERCENTAGE FREQUENCY OF OCCURENCES (%)
<i>Lactococcus lactis</i>	1	7.7
<i>Staphylococcus aureus</i>	2	15.4
<i>Bacillus megaterium</i>	3	23
<i>Bacillus cereus</i>	2	15.4
<i>Alcaligenes faecalis</i>	1	7.7
<i>Citrobacter freundii</i>	2	15.4
<i>Bacillus subtilis</i>	1	7.7
<i>Aeromonas hydrophila</i>	1	7.7
Total	13	100%

Serial Dilution

The result of serial dilution showed the Serial dilution factor indicating the identified bacteria colony . **table 3**

**Table 3. Serial dilution factor with Standard deviation.**

Sample	Dilution factor 10 <sup>-5</sup>	10 <sup>-4</sup>	×	×	Colony forming unit (C.F.U)	Standard deviation
<b>A</b>	Too numerous to count	168	168	84	16.8	42
<b>B</b>	Too numerous to count	316	316	158	31.6	79
<b>C</b>	Too numerous to count	180	180	90	18.0	45
<b>D</b>	Too numerous to count	224	224	112	22.4	56
<b>E</b>	240	80	320	160	32.0	80

Key = x= Total number of dilution factor,  $\bar{x}$  = mean, C.F.U = Colony forming unit. SD= Standard deviation

### Identification of fungal Isolates

The isolates obtained were identified as indicated in **Table 4** which include *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus oryzae*, *saccharomyces cerevisiae*.

**Table 4. Identification of fungal organisms from Cassava samples.**

Identified organism	SAMPLES				
	A	B	C	D	E
<i>Aspergillus niger</i>	-	-	-	+	+
<i>Aspergillus fumigatus</i>	-	-	-	+	+
<i>Aspergillus oryzae</i>	-	+	-	-	-
<i>Saccharomyces cerevisiae</i>	+	-	+	+	-

Key= positive (+), Negative (-).e

The result is given in **Table 5** the Percentage Frequency of Occurrence of fungal isolates with *Aspergillus niger* having (38.5%), *Aspergillus fumigatus* (2.3%), *Aspergillus oryzae* (7.7%), and *saccharomyces cerevisiae* (30.8%).

**Table 5: Percentage Frequency of Occurrence.**

Fungi Identified	Number of Occurrence	Percentage Frequency of Occurrences (%)
<i>Aspergillus niger</i>	5	38.5
<i>Aspergillus fumigates</i>	3	2.3
<i>Aspergillus oryzae</i>	1	7.7
<i>Saccharomyces cerevisiae</i>	4	30.8
<b>Total</b>	13	100

Cultural and Morphological characteristics Identification of fungal Isolates **Table 6.**

**Table 6: Morphological characteristics of Fungal Isolates**

Samples	Cultural	Microscopic	Isolates
A	Dark-brown to black	The colony usually contains black conidiophore. Conidial heads, radiate. Conidiophore stipe smoothwalled, hyaline but often in brown colour. Vesicles globose to sub-globose.	<i>Aspergillus niger</i>
B	Dark-green to blue	Colonies have dense felt of dark-green conidiophores. Conidia heads typically radiate latter splitting in several loose columns, yellow-green becoming dark yellow-green. Sclerotia are brown to black.	<i>Aspergillus fumigatus</i>
C	Yellow white	Colonies have yellow-green conidiophores. Conidiophores have stipes with smooth-walled hyaline but often in brown color.	<i>Aspergillus oryzae</i>
D	Creamy white	Colonies extent quickly and developed within three days. They have flat, moist, glittering or dull, and cream in color. Blastoconidia are present.	<i>Saccharomyces cerevisiae</i>

## IV. DISCUSSION

The series of microbial examination of the cassava samples indicated that all the samples were infected by microbes. This could be as a result of frequent touching of the samples by both buyers and sellers thereby

contaminating the food items. This is in line with the findings of (Akinsanya, *et al.* (2013) who stated that sale and distribution and marketing of cassava products such as boiled cassava, abacha, garri and akpu in local markets is associated with unwholesome and unhygienic practices which may lead to microbial contamination due to transfer of microbes from dirty hands and utensils and frequent visits by animals and fomites which increases microbial load of these products. The findings also showed the percentage prevalence of the bacteria and fungi agents *Bacillus megaterium* and *Aspergillus niger* had the highest occurrence of 23.00% and 38.5% prevalence while *Staphylococcus aureus*, *Citrobacter freundii* *Bacillus aureus* had a percentage occurrence of 15.4% respectively is an indicative that they could cause danger to health of the consumers as majority of those microbial organisms are pathogenic. The result from this study also revealed that bacterial and fungal isolates identified are commonly present as contaminants generated from human skin, cooking utensils processing equipment, the environment and water (Omemu and O. Faniron, 2011). Odetunde *et al.*, (2014) observed that pathogenic organisms in foods may indicate that such foods were exposed to conditions favorable for their introduction and growth.

According to Ogiehor and Ikenehomeh (2003) the microbial contamination of traditionally processed food products were likely from dirty containers bags, measuring and transport devices, dirty water, sneezing, coughing, talking dust raised by passers-by and vehicles, dirty environment and other unhygienic habits of the processors. Therefore the presence of *lactococcus*, *Staphylococcus aureus*, *Bacillus spp*s identified in this study might have been attributed to come from the mouth, nose and skin of the food handlers with the implication that such food products might not be safe for human consumption. Isolation of the of the eight pathogenic organisms in the processed cassava meal products sold within Sokoto central market corroborated the findings by Ukah and Imere (2017) in their study on the impact of processing environment on the microbial quality of garri processing in Benin city, Edo State. WHO (2005) stated that pathogenic organisms such as *Staphylococcus aureus*, *Escherichia coli*, *Salmonella enteritidis*, *Klebsiella pneumonia* have been known to cause gastro intestinal infections, sporadic and epidemic diarrhoea, food poisoning, typhoid fever and food intoxication among other food borne disease.

## V. CONCLUSION

The dictecton and isolation of bacteria and fungi from apparently cooked cassava sold in the market posed a dangerous threat to people's health. Hence, the need to carry out sensitization campaign on proper food hygiene for our safety.

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