BACTERIOLOGICAL ASSESSMENT OF GARRI SOLD IN AUCHI METROPOLIS

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CERTIFICATION

This is to certify that this project work "Bacteriological Assessment of Garri Sold In Auchi" Metropolis was carried out by ABUDUKARIMUHAFSAT with matriculation number AST/2382010442 and ABUDUAISHATU with matriculation number AST/2382070415 in the department of biological science laboratory, Auchi polytechnic, Auchi. In partial fulfillment of the requirement for the award of Higher National Diploma (HND) in science laboratory technology (Microbiology option).

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DEDICATION

Wededicate this work to the God almighty whose boundless infinite love, goodness, guidance, favour, gave us a sense of knowledge and wisdom to successfully complete this project work.

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We give thanks to God Almighty the maker of the universe for our life and also making us to accomplish ourgoal in life, also for giving us the strength and determination to put this project work together.

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ABSTRACT

This study was on bacteriological quality of Garri sold in Auchi metropolis. The Garri samples were collected aseptically from two different markets in Auchi. The samples were taken to the laboratory immediately and were analyzed using standard micro biological and biochemical methods.Staphylococcus species, Micrococcus species and Bacillus species were the bacteria isolated from the sample. The total bacterial count ranged from 1.1×10^{2} *to* 1.2×10^{4}.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Food security both in developed and developing countries has been a growing concern that has led to an unprecedented global interest in Agriculture (Orji *et al.,* 2016) because of the alarming concern of disease outbreak caused by consumption of contaminated food and food products. Market vending has become an important public health issue and a great concern to everybody. This is due to the widespread of food borne disease associated with Garri handlers who lack adequate understanding of the basic food safety issues (Ghosh *et al.,* 2015).Open bowl display of this fermented cassava (Garri) is perceived to be a major public health risk due to lack of basic infrastructure (Rane *et al*., 2015). Various studies have identified the source of food safety issues involved in market sales of Garri to be microorganisms (Ghosh *et al.,*2015).

The processing of cassava tuber to Garri and its handling involves different stages, at each stage there is a level of contamination (Adejumo et al., 2015). The quality of the product depends on the management of each stage of the processing and handling. The processing of cassava into Garri usually takes three to five days both

at household and factory level. The unhygienic practices carried out in local markets in Nigeria is associated with practices that lead to microbial contamination due to deposition of bio aerosols on exposed products, which may cause food poisoning and may lead to disease outbreak as a result of these contaminated food products. Today Cassava remains one of the most common source of dietary food energy to people especially in Africa.

Recently in Nigeria, the Federal Government launched an agricultural transformation agenda to promote agriculture as a business to integrate the agricultural value chains and as a possible key way of driving Nigeria's economy (Agah *et al.,* 2016). Cassava supplies about 70% of the daily calorie to over 50 million of people worldwide (Oluwole *et al.,* 2014). It can be processed into bread, Garri, flour etc. (Orji *et al.,* 2016) (Adejumo and Adebayo 2015). Among the proceeds of cassava, Garri is an important by product that is commonly consumed in Nigeria because of its ready to eat nature (Orji *et al.*, 2016) (Adejumo and Adebayo 2015), it is the major source of energy and fiber (Ogiehor *et al.*,2017). Garri can be produced locally by fermentation of peeled cassava tuber in Nigeria and other parts of the world (Ray and Sivakumas 2018). The unhygienic handling and poor sanitary measure are obviously been observed between the last stages of production and could constitute serious health implication as many chances have been given to contaminate the food products. The dust being raised by the breeze,

storm, passing by vehicles and every other form of air movement bring solid particles and heavy metals into the fermented cassava (Garri). Heavy metal contents in food has a limit, if these limit are exceeded, it could cause harm to the human body. It has also been reported that Garri sold in markets contain high load of microorganism (Ogiehor *et al.*,2017) which might cause economic loss and food borne illness and public health threat as a result of these contaminations. The patronage of many consumers could constitute serious health implication as many chances have been given to contamination by organism of epidemiological importance such as *Salmonella, Escherichia coli, Klebsiella, Staphylococcus aureus, Staphylococcus epdidermidis Cryptosporidium, Campylobacter, L.grayi, L ivanovii.* Garri and it ready to eat nature has made it a common practice in Nigeria especially among students to eat Garri as snacks without considering the bacteriological implication (Egbuobi *et al.*,2015).

1.2 Objectives of the Study

The specific objectives are;

1. To isolate and identify bacteria from Garri sold in Auchi metropolis, Nigeria.
2. Characterization and identification of isolate.
3. To determine the Pathogenecity of the isolate.

1.3 Justification of the Study

There is limited information to the assessment of some bacteria associated with Garri sold in Nigeria (Olapade *et al.*, 2014) and even the study area. Studies have not been reported, and hence this study provides information on bacteriological quality of Garri sold in Auchi metropolis, Edo State.

1.4 Statement of the Problem

The sale of Garri in the local markets in Nigeria is associated with practices such as open display in bowls and trays, open buckets and mats at points of sale and the use of bare hands in handling and selling of cassava (Garri). These unhygienic practices, may lead to microbial contamination and can cause deterioration in food quality and spoilage, food borne illness and may pose a threat to public health. Food security has been a major challenge to the world populace over the last few centuries because of the alarming concern of disease outbreak caused by consumption of contaminated food and food products.

1.5 Area of study

Auchi is the second largest city in Edo State, Nigeria after Benin City, the capital. Auchi is located in Etsako West Local Government Area of Edo State and is also the headquarter of the local government area which comprises Auchi, Uzairue,

South-Ibie, Agbede and Anwain-clan. During the British colonial rule, it was the headquarter of kukuruku division the administrative headquarter of five district.

The population grew to 42,628 by 1952, including people from many Nigerian tribes. As of 2015-2016, the population was 152,652. Auchi has in recent years been amongst the fastest growing cities in Nigeria.

CHAPTER TWO

LITERATURE REVIEW

2.1 Cassava

Cassava (*Manihot esculenta*) is a woody shrub native to South America of the spurge family, Euphorbiaceae. Although it is a perennial plant, cassava is widely cultivated as an annual crop in tropical areas. The cassava root is long and tapered; it has a firm homogenous flesh enclosed in a detachable rind and about 1mm thick, rough and brown on the outside. The fleshy part may be chalk-white or yellow in color on the outer part with woody vascular bundle extended along the root axis. It is the chief source of dietary food energy for the majority of the people living in the low land tropics, and much of the sub-humid tropics of West and Central Africa. It supplies about 70% of the daily calories of over 50 million people (Oluwole *et al.,* 2014) in Nigeria and about 500 million people in the world (Abu *et al.*,2016).

The competing needs for cassava cut across both humans and animals. It is fast becoming a popular raw material in industrial production and is now a preferred material for making biofuels. Cassava is God's gift to the tropics because it can grow in poor soil with inadequate rainfall. The starchy roots of cassava are major

source of food for more than 700 million people all over the world. It ranks third in order of staple food crops in developing countries after rice and maize. It has universal applications. Nigeria is the world largest producer of Cassava (Adeniji *et al.*,2015). We produce over 41 million metric tons per annum and we are followed by Brazil, Thailand, Zaire (now Democratic Republic of Gongo), and Indonesia. Nigeria has tried to expand the local cassava business through the Composite Flour Initiative and the Cassava Empowerment Fund but so far, they have attained little or no success (Knipscheer *et al.*,2017).

Source: Cassava Farm

2.2 What is Garri?

Garri is a creamy – white, granular flour with a slightly fermented flavor and slightly sour taste made from fermented, gelatinized fresh cassava (*Manihot*

esculenta Crantz) tubers. Garri is widely known in Nigeria and other West African Countries. It is commonly consumed either dry or soaked in cold water with sugar, coconut, roasted groundnuts, dry fish as compliments or as a paste with hot water called "Eba" which is eaten with varieties of African Soup. It can also be taken soaked or dry without any compliment. When properly stored, it has a shelf – life of six months or more (Amadi and Adebola, 2012).

Garri is dry, crispy, creamy-white and granular. It is a dehydrated, cassava product. It is classified or grouped based on texture, length of fermentation, region or place where it is produced and color imparted by the addition/non-addition of palm oil (Abu *et al.*,2016). It has a high swelling capability and can absorb up to four times its volume in water (Osungbaro *et al.*,2014). Garri is an important by-product of cassava being an important item in the menu of most Nigerians. It is particularly popular because of its ready-to-eat nature (Abu *et al.*,2016). Garri is a good source of energy and fiber. Other nutrients are also present in marginally nutritional significance. Cassava for Garri production is harvested manually in the farm with the aid of cutlasses, hoe and flat iron sheet (digger), which occasionally inflicts various degrees of injury on the root tubers. After harvesting, the root tubers are hauled to the market where they are heaped in 20s, 40s, 50s, and 100s for sales under humid and warm topical conditions. These practices predispose the root

tubers to contamination and infestation by various microorganisms (Ikegwu *et al.*,2018).

Source: (Ikegwu *et al.*,2018).

2.3 Processing of cassava into Garri

Garri is traditionally made at home in Africa using mechanized means, Garri can also be made into commercial quantities. In processing cassava into Garri, the raw cassava must undergo a series of processing steps which includes:

Step 1: Harvest and sort your cassava to get the best

This is the last step in cassava farming and the first step in the process of Garri production. After harvesting your cassava from the farm, select only healthy whole roots that are appropriate for producing Garri. Because there is a need to select healthy cassava tubers, you need to sort your roots and pick the best ones. After the

sorting, transport the tubers to the cassava processing plant. Harvested cassava to be processed into Garri.

Step 2: Peel the Cassava Roots

When you reach the cassava processing plant, peel the cassava root using a knife.

You can also peel the roots before taking them to the cassava processing plant, especially if the processing plant has a good waste disposal system. Some processing plants have complete cassava processing machines including peelers which you can use to peel larger quantities of cassava.

Step 3: Wash and clean the cassava root

Wash the fresh cassava roots in clean water to remove excess mud and sand.

Use a clean sponge or scourer to wash the cassava clean, removing extra peel, spots, or dirt. There is a special Garri processing machine for this step too. This step of Garri processing is very important so that the quality of the final Garri is without sand.

Step 4: Grate the Cassava Roots into Mash

The need to grate cassava roots into mash is to remove some cyanide from the roots in the process. In ancient times, people use a rough metal sheet to grate the cassava by hand. They usually sustain severe injuries on their fingers.

Nowadays, there is more efficient cassava processing machinery which is safer and easier to operate. There are also locally fabricated Garri processing machinery in Nigeria which you can get at an affordable price. However, the grating machine must be one with a stainless steel grating drum. If you use a grater that is in any way less than stainless steel, you may be risking introducing rust and contaminating your product. To grate the cassava using cassava grating machinery, load the roots carefully onto the engine while its running to grate it into a mash. Collect the mash from the output port of the machinery into a clean bowl. The next step is to pour it into a clean polythene sack and allow it to ferment and drain.

Note:

NEVER Ever try to use your hands to force the roots deeper into the grater while it is still running!

Step 5: Ferment the Cassava Mash to Remove Hydrocyanic Acid

The essence of fermenting the cassava mash is to get rid of the hydrocyanic acid (HCN) which is natural in cassava tubers. You must control the fermentation process properly because if you leave it to ferment for a very short period, it will result in incomplete detoxification and a bland product. If you leave the cassava mash to ferment for too long a period, it will give the product a strong sour taste. So, both over-fermentation and under-fermentation of Garri will greatly affect the texture of the final Garri.

Step 6: Press the Cassava Sack to Drain the Water in the Garri

Before going into sifting and frying the Garri, you need to drain the water completely. You know you can't achieve that by squeezing the bag of Garri with your hands. So, you have to load the bags directly onto a hydraulic press and tighten them very well. Some people, especially those in rural areas, use wood and strong chords to tie the bag of Garri while in some places they use heavy stone on the bag of Garri to increase the pressure. This is a very slow and unhygienic method of draining water from your Garri. It is not a good method for a commercial Garri producer. The key thing to note in this step is to find the perfect moisture balance for the Garri. If after pressing the Garri mash it is too wet, it will get lumpy during roasting. Also, if it is too dry, it will be too floury and dust like. How to know that the Garri is ready for frying is that it will form a firm wet cake inside the bag. Another way is when no more water comes out of the bag, it means that the Garri is ready for the next step. You will need to break it apart so that it comes out in smaller blocks which you can crush with your hands for sifting.

Step 7: After Draining the Garri Mash, Sift the wet Cake Into Grits

In this step, all you have to do is to take out the wet Garri cake and break it down with your hands. What you will have will be grits of Garri. Using a sifter, sift the Garri grits to remove the lumps and fibrous roots it contains. The aim is to obtain a high-quality product with uniform size granules

Step 8: Fry or Roast the Grits to Form Edible Garri

To fry the Garri, gently spread some quantity of the grits into a frying pan or a Garri fryer made of stainless steel material. A Garri fryer can make use of electricity, natural gas, firewood, or charcoal as the heat source. Whatever the source of heat is does not matter, what matters is the consistency of the temperature. Use a temperature of 80 ºC/175 ºF to fry the Garri until it is okay for removal.

If you use lower temperatures, the product will simply dry up slowly and produce a dry white powder. Also, if the temperature is too high, it will result in the charring of the product and it will make it stick to the roasting pan. One thing you must do is always stir the Garri until it is ready for evacuation from the fryer. Doing this will make the heat spread uniformly throughout the Garri roasting process. How to know that your Garri is ready after roasting? Scoop a little quantity of the Garri out of the fryer let the hot Garri cool down a little handle it with your hand and judge; if it is dry and crispy, then it is ready for evacuation, otherwise, continue frying.

When the Garri finally gets the right texture, scoop everything out of the fryer and repeat the process of frying another quantity of Garri until you finish everything.

Step 9: Spread the Garri in a Thin Layer and Allow it to Cool

After roasting the Garri, spread it in a thin layer on a platform that is higher than ground level and allow it to cool.

The reason for spreading the Garri on a high platform is to prevent sand from entering it.

Also, when you are cooling your Garri, make sure the environment does not have high humidity. Garri is hygroscopic, meaning it absorbs moisture from the air. As a result, you must ensure that the humidity of the environment is conducive and dry enough for the Garri.

Step 10: Sieve or Grind the Garri to Break Larger Granules

As soon as the Garri becomes cool enough, use a sieve to remove the larger granules in the product. Alternatively, you can use a grinder to break down the larger granules into smaller ones. Some people just pack everything the way it is.

Step 11: Pack the Garri in Airtight Bags and Store Properly

The final stage of this guide for cassava processing into Garri. Because of the hygroscopic nature of Garri, it should be packed in airtight and moisture-proof

bags. This is very important especially in areas of high humidity, to prevent mold growth. Pour the fine Garri into a plastic lining inside a woven polythene sack and store it in a cool dry place.

Souce:https://agro4africa.com/cassava-processing-how-to-process-raw-cassava-into-Garri/

2.4 Types of Garri

There are different types of Garri, depending on how is processed, and the region of Africa where it is produced. The Standards Organization of Nigeria classifies Garri into:

a. **Red Garri:** This is the type of Garri commonly found in the Mid – Western part of Nigeria. It is also called Bendel Garri. It is made exactly the way described above, but for the addition of red palm oil after grating the cassava and the Garri is allowed to ferment for two to three days also. Adding palm oil to the Garri further helps to reduce the cyanide content and gives it a unique flavour.
b. **White Garri:** Same as Bendel Garri, left to ferment for two to three days as well, but red palm oil is not added during processing.
c. **Ijebu Garri:** Ijebu Garri is made same way too, but allowed to ferment for up to seven days. No palm oil is added. It is also fried to become much crisped. It characteristically has a very sharp tasted and less starchy. Many

people from the Western part of Nigeria love this and find it great for "soaking".

2.5 Microbial Quality of Garri

The fermentation of cassava to produce Garri provides an enormous scope of value addition and preserves this starchy food in a wide diversity of flavours, aromas and textures that enrich the human diet and helps to ensure distribution and storage of the product without the need for refrigeration. However, post-process problems of Garri still persist and include loss of microbial stability and spoilage during storage, distribution and marketing. In Benin, the sale and distribution of Garri in local markets is associated with practices such as display of product in open sacs, bowls and mats at points of sale and the use of bare hands during handling and sales. These unhygienic practices which may lead to microbial contamination due to deposition of bio aerosols on exposed products, transfer of microbes from dirty hands and utensils and frequent visits by animals and fomites (which may carry infectious agents), can contribute to the post – process problems of this product. Previous reports have revealed high a vast array of microorganisms in market samples of Garri. The microorganisms isolated from these samples include: *Bacillus spp., Pseudomonas spp., Clostridium spp., Salmonella spp., Klebsiella spp., Aspergillus spp., Penicillium spp., Rhizopus spp., Fusarium spp., Cladosporium spp.,* etc. (Bartram, 2013) Heterotrophic plate counts and drinking-

water safety: the significance of HPCs for water quality and human health. WHO Emerging Issues in Water and Infectious Disease Series. London, IWA Publishing. These microorganisms can cause deterioration in food quality and spoilage, serious food borne illnesses and may pose a threat to public health. Moreover, the source of these microbial contaminants may also be a portal for contamination by more potent pathogenic microbes which may cause an epidemic considering the popularity of the food product.

2.6 Effect of Some Microbes Found in Garri on the Human Body

2.6.1 Bacillus spp. are large (4-10μ), Gram-positive, strictly aerobic or facultative anaerobic encapsulated bacilli. They have the important feature of producing spores that are exceptionally resistant to unfavourable conditions. *Bacillus spp.* Are classified into the subgroups *B. polymyxa*, *B. subtillis* (which includes *B. cereus* and *B. licheniformis*), *B. brevis* and *anthraicis*. (Bartram, 2013).

Human Health Effects

Although most *Bacillus spp.* are harmless, a few are pathogenic to humans and animals. *Bacillus cereus* causes food poisoning similar to staphylococcal food poisoning (Bartram, 2013). Some strains produce heat-stable toxin in food that is associated with spore germination and gives rise to a syndrome of vomiting within 1-5hours of ingestion. Other strains produce a heat-liable enterotoxin after

ingestion that causes diarrhea within 10-15h. Bacillus cereus is known to cause bacteremia in immunocompromised patients as well as symptoms such as vomitingand diarrhea. Bacillus anthracis causes anthrax in humans.

2.6.2 Klebsiella spp.are Gram-negative, non-motile bacilli that belong to the family *Enterobacteriaceae*. The outermost layer of *Klebsiella spp.* Consist of a large polysaccharide capsule that distinguishes the organisms from other members of the family. Approximately 60-80% of all *Klebsiella spp.* can also be identified as pathogens (Ainsworth, 2014).

Human Health Effects

Klebsiella spp. have been identified as colonizing hospital patients, where spread is associated with the frequent handling of patients. Patients at highest risk are those with impaired immune systems, such as the elderly or very young, patients with burns or excessive wounds, colonization may lead to invasive infections. On rare occasions may cause infections, such as destructive pneumonia (Ainsworth, 2014).

2.6.3 Salmonella spp. Belong to the family of Enterobacteriaceae. They are motile, Gram negative bacilli that do not ferment lactose, but most produce hydrogen sulfide or gas from carbohydrate fermentation. (Ainsworth, 2014).

Human Health Effects

Salmonella spp. Infections typically cause four clinical manifestations: gastroenteritis (ranging from mild to fulminant diarrhea, nausea and vomiting), *bacteraemia* or *septicaemia* (high spiking fever with positive blood cultures), typhoid fever / enteric fever (sustained fever with or without diarrhea) and a carrier state in persons with previous infections (Ainsworth, 2014).

d. Aspergillus spp. Aspergillus species are highly aerobic and are found in almost all oxygen-rich environments, where they commonly grow as molds on the surface of a substrate, as a result of the high oxygen tension. Commonly, they grow on carbon-rich substrates like monosaccharaides (such as glucose) and polysaccharides (such as amylose). *Aspergillus spp.* Species are common contaminants of starchy foods (such as Garri), and grow in or on many plants and trees (Ainsworth, 2014).

2.7 Mechanization of Garri Production

Mechanization of Garri production is the use of machines, either wholly or in part, to replace human or animal labor in the production of Garri, unlike automation, which may not depend at all on a human operator, mechanization requires human participation to provide information or instruction. It implies the use of machinery more complex than hand tools and would not include simple devices such as an un-

geared horse or donkey mill. Devices that cause speed changes or changes to or from reciprocating to rotary motion, using means such as gears, pulleys or sheaves and belts, shafts, cams and cranks, usually are considered machines.

Equipment for rapid processing of cassava has been used in Nigeria for over 20 years (Amadi and Adebola, 2012). The stages in the processing of cassava include: peeling, washing, grating, dewatering, granulating or sieving, roasting, cooling and packaging. Peeling is sometimes done manually because cassava is bulky and irregular in shape with various peel thickness. Mechanical peeling results in heavy losses. Washing are also manual for convenience and to reduce cost. There are many models of grater. Using electricity, diesel or petrol motor, the grating surfaces are made from iron sheet, galvanized iron or stainless steel; the first two being rust-prone. Low cost and low energy graters are available in the market; women processors use them. Sieving or granulating is manual and is done on raffia or metal sieves. There are metal sieves which can be used while standing or shaken mechanically (James *et al.,* 2012). Rapid removal of water from fermented pulp lasts from 30mins to 2hr and is achieved by using hydraulic jack or screw press. Rotating over heat 22-600 is preferably carried out in a cast-iron pan or an assortment of trays (Ayehu *et al.,* 2014). Rotating-drum roasters do not produce Garri of good quality because such devices do not mix and roast well (Amadi and

Adebola, 2012).The cooling of Garri after roasting takes place on suitable trays and the product may be packaged in thick polythene bags.

2.8 Nutritional Value of Garri

Garri is highly rich in starch and fiber content. It is also noticed to contain some amount of proteins, calories, sodium, fat, potassium, copper, iron magnesium, manganese, little calcium selenium, zinc and some essential vitamins like vitamins B6, C and E. The fiber content of Garri makes one to feel full when it is been consumed, and it is very helpful in preventing ailments such as constipation and bowel diseases. It provides us with energy because of its high starchy content.

Red or yellow Garri contains fats and oils, which are great sources of additional nutrients and health benefits.

The major health benefits of Garri are that it serves as a complementary food to balance our diet.For example, Garri (eba) is being eaten with soups such as vegetables, meats, fish, fats and oil, minerals etc., and they provide various nutrients that make the meal to be a balanced diet. (Health and Nutrition, 2017).

2.9 Factors Involve in Contamination of Garri During Production

Garri processors are involved in practices which contribute negatively to the microbial quality of the processed Garri. Some of the practices include; burying of

basin inside the ground to serve as a discharged point for the grinded cassava paste from the machine: This practice enhances soil particles and debris to fall directly into grinded paste thereby enhancing microbial contamination. The grinding machine is also characterized by visibly unwashed left over paste. This serve as a source of contamination to fresh cassava paste (Lawani *et al*., 2015).

Keeping of dried cassava paste sack on bare ground. There is the possibility of soil microbes finding its way through the sack into the dried cassava paste. The floor of the manual presser also having direct contact with the ground enhances microbial contamination. Unskilled nature of the Garri producers introduces contaminants to their products. Sitting of the cassava effluent site close to the processing site. Poor source of water and dirty processing environment (Ogiehor and Ikenebomeh, 2015). Dirty environments attributed to markets and indiscriminate dumping of refuse around the markets where Garri is sold is another major source of contamination.

CHAPTER THREE

MATERIALS AND METHOD

3.0 Materials

The materials used for this study include samples of Red and White Garri from Uchi and Jattu Market, nutrient agar, petri dishes, 2ml syringes, cotton wool, foil paper, microscope, weighing balance, distilled water, beaker, masking tape, oven, autoclave, incubator, fringe, test tube, wire loop, slides,Bunsen burner, colony counter, conical flask, hand gloves.

3.1 Collection of Sample

The White and Red Garri were collected from Uchi and Jattu market, Auchi, Edo State. All samples were transported in sterile nylon bags to the laboratory.

3.2 Sterilization of Apparatus

All glasswares were washed with detergent, rinsed with distilled water, dry clean and sterilized using an oven for 1hour.

3.3 Preparation of Nutrient Agar

Weigh 14 grams of nutrient agar in 500ml of distilled water, swirl and mix, then sterilized by autoclaving for 15 minutes at 121^0C.Allow to cool to 47^0C before pouring.

3.4 Preparation of Stock Solution

1g of the four samples of Garri were transferred into sterile beakers each and labeled accordingly. 9ml of distilled water was measured using syringe into each beaker containing the samples and mixed properly.

3.4.1 Serial Dilution

5Folds of serial dilution was used. A sample with five test tubes were labelled W1,W2,R3,R4. 1ml of the starting sample was added to 9ml dilution blank tube 1. This is then followed by the same procedure, where 1ml from tube 1 is added to 9ml of tube 2, 1ml from tube 2 is added to 9ml from tube 3, and so on untilit get to the 5^{th} tube using syringe.Then tube 1 and 3 was used for the pour plating.

3.5 Method of Inoculation

Using pour plate,0.1ml of folds 1 and 3 from each samples were injected into sterile petri dishes. The mouth of the lid containing molten media was opened and flamed,and about 15ml of sterile molten media was poured at the appropriate

temperature above the sample. The lid of the plate was closed , then the sample and the media was mixed properly by gently swirling the plate . The plate is generally swirled in an “S” or 8 shape. The mixture is left to solidify, and then incubated using an incubator for 24hours at 37^{o}C. After incubation, there was growth in the medium.

3.5.1 Plate Count

After 24hours of incubation, with the aid of colony counter,the colonies were counted and expressed as (cfu/g i.e colony forming unit per gram)

3.6 The Isolation of Pure Culture

Distinct colonies observed for each plates where sub-culture into fresh nutrient agar petri dish respectively. This was done using streak method. In this method, a sterile wire loop was used to transfer a loop full of each colony aseptically and streaked on a fresh media. The fresh media plates were now incubated in the incubator at 37^{0}C for 24 hours. After incubation, the pure culture of the bacterial that grew was now subjected to characterization.

3.6 Characterization and Identification of Bacteria

In order to identify the bacteria isolate, morphological and biochemical test were carried out. The identification was based on cultural and microscopic characterization. Microscopy was focused on the shape, colour and arrangement.

3.7 Gram Staining

Gram staining is a method of staining used to classify bacteria species into two large groups which are, gram positive bacteria and gram negative bacteria.

Usingeach old culture sample of 24hours, a smear was prepared on a clean slide each using a sterile wire loop. The smear was heat fixed by passing the slide over a flame in order to allow the organism to stick to the slide so as to avoid washing off during subsequent process. After fixing, the smear was flooded with a drop of crystal violet(primary stain) for one minute, after which it was rinsed off with distilled water. The slide was then flooded with gram iodine (mordant) for one minute and rinse with water. A drop of decolorize agent (Acetone) was added and rinse immediately, the acetone increases the permeability of the cell membrane. A drop of counter stain,(safranin) was applied for one minute and rinsed off. the slide was allow to air dry and observed under the microscope with oil immersion objective (100x) lens. The organisms that pick up the colour of the crystal violet

(purple colours) were grampositive organism while the gram negative organism were those that retain the colour of safranin which is pink or red.

3.8 Biochemical Test

3.8.1 Manittol Salt Agar Base

Measure 27g of MSAB into a conical flask, Add 250ml of distilled water and cover with foil paper. Autoclave for 30 minutes. After autoclaving, The MSAB was poured into a petri dish and allow to cool. The petri dish was divided into four places using a marker and label W1, W2, R3, R4 at the back, using a sterile wire loop, an isolate was picked from each culture medium and streak in the appropriate labelled parts of the petri dish containing the MSAB and inverted . The petri dish was placed in an incubator, in an inverted position,and left for 18 hours. When the background changes to pink, it is positive but if it remain the same, is negative.

3.8.2 Triple sugar iron agar (Orange in colour)

Divide 64.52 by 4 =16.13

Pour 16.1 of TSIA in a conical flask and add 250ml of distilled water, stir using spatula, cover with foil paper. Autoclave for 30 minutes. After autoclaving,it was allow to cool. The TSIA was poured into four test tubes,labelledand slantly

positioned. A samplewas picked using a sterile wireloop and inserted into the TSIA test tube labeled, the test tubes were covered with foil paper and incubate for 24hours.

3.8.3 Simmon citrate agar (green in colour)

Pour SCA in the four test tube labell with their samples and keep it slant, then cover with foil paper, using a sterilize wireloop, pick the samples and put on the labell test tube, cover with foil paper and put in test tube rack, incubate for 24 hours.

Method for Catalase Test

24 hours old culture was used for the test. The test was performed by emulsify few colonies of the organism on a clean slide using wire loop to pick and put on the slide heat fix, a drop of hydrogen peroxide on each of the samples on the slide. The production of bubbles indicate positive and absence of bubble indicates negative.

Aim

To demonstrate the presence of catalase in an organism.

Principle

Catalase is an enzyme, which is produced by microorganisms that live in oxygenated environments to neutralize toxic forms of oxygen metabolites and H_2O_2.

Materials Required

24 hours old bacterial culture, glass slide, petridish, 3% H2O2, applicator sticks

Procedure

a. Asmall amount of sample W1, W2, R3, R4 was transferred to a surface of clean, dry glass slide using a loop .
b. A drop of 3% H2O2 was placed on the slide and mix.
c. A positive result is the rapid evolution of oxygen (within 5-10 s) as evidenced by bubbling.
d. A negative result is no bubbles or only a few scattered bubbles.
e. Dispose of your slide in the biohazard glass disposal container.

3.8.5 Coagulase Test

This test was to demonstrate the presence of enzyme coagulase. Coagulase is on enzymes that causes plasma to clot by converting fibrinogen to fibrin.

Aim

To distinguish coagulase producing Staphylococcus aureus from other species of Staphylococcus

Principle

Staphylococcus aureus is known to produce coagulase, which can clot plasma into gel in tube or agglutinate cocci in slide. This test is useful in differentiating *S. aureus* from other coagulase-negative staphylococci.

Materials Required

EDTA anticoagulant human plasma, clean glass slide, test tubes, pipettes, distilled water and inoculation loop.

Procedure

Slide Coagulase Test: Dense suspensions of Staphylococci from culture are made on two ends of clean glass slide. One should be labeled as “test” and the other as “control”. The control suspension serves to rule out false positivity due to auto agglutination.

3.6.6 Indole Test

This is used in differentiation of gram negative bacilli.A sterilized wire loop was to pick each sample W1, W2, R3, R4 and inserted into the test tubes containing peptone water and covered with cotton wool, then incubated for 24hours.

Aim

To determine the ability of microorganisms to decompose the amino acid tryptophan to indole

Principle

Tryptophan an essential amino acid oxidized by some bacteria by the enzyme tryptophanase resulting in the formation of indole, pyruvic acid and ammonia. In this experiment, the medium contains the substrate tryptophan which is utilized by the microorganisms.

Materials Required

15 ml test tubes, bacterial culture, peptone water, Kovac's reagent Procedure

a. The peptone water tubes were inoculated with bacterial broth culture using sterile needle .
b. An uninoculated tube was kept as control.
c. Both tubes were incubated at 37°C for 24-48 hours.

d. After proper incubation, 1 ml of Kovac's reagent was added to both tubes including the control.
e. The tubes were shaken gently after an interval for 10 – 15 minutes.

3.6.7 Methyl Red Test

Aim

To determine the ability of microorganism to oxidize glucose with the production and stabilization of high concentrations of acid end products

Principle

All enteric organisms oxidize glucose for energy production and the end products of this process will vary depending on the specific enzymatic pathway present in the bacteria.

Procedure

a. Using sterile technique experimental organisms were inoculated into appropriately labeled tubes containing MR broth by means of loop inoculation.
b. Uninoculated tube was kept as control
c. Both tubes were incubated at 37°C for 24-48 hours.

d. After proper incubation 5 drops of MR indicator was added to both tubes including control.
e. It was mixed well and colour was observed.

Citrate Utilization Test

Aim

To determine the ability of a microorganism to utilize citrate as the sole source carbon and as energy

source for the growth and ammonium salt as a sole source of nitrogen

Materials Required

Bacterial broth, Simmons Citrate Agar Slants, Inoculation Loop

Procedure

a. Using sterile technique Simmons citrate agar slant was inoculated with the test organism by means of a stab and streak inoculation.
b. An uninoculated tube was kept as control.
c. Both tubes were incubated at 37°C for 24 – 48 hours and was observed

3.9 PRECAUTIONS

1. It was ensured that all glasswares and materials used were sterilized appropriately.It was also ensured that aseptic conditions were maintained throughout the process to avoid external contamination of the food samples.
2. Adequate timing and required temperature were used as required at different stages.
3. Each of the equipment used was checked to be in good working condition before use.
4. It was also ensure that proper documentation was done at each stage of reading.
5. It was ensure that laboratory coat, handgloves, nose mask was worn before carrying out any test.

CHAPTER FOUR

RESULT AND DISCUSSION

4.1 RESULT

The table below shows the result of the bacteria isolate present in garri.

Table 1: Total number of viable aerobic bacterial count.

Samples	**Dilution factor**	**Number of colonies on agar plate**	**Total numbers of colony forming unit (cfu/ml)**
W1	10^{-1}	11	1.1×10^{2}
W2	10^{-3}	8	8.0×10^{3}
R3	10^{-3}	12	1.2×10^{4}
R4	10^{-1}	20	2.0×10^{2}

Table 2: Cultural, Morphological and Biochemical Characteristic of Bacteria

characteristics	**Isolate 1 W1**	**Isolate 2 W2**	**Isolate 3 R3**	**Isolate 4 R4**
Colony Morphology	Light yellow	Light cream	Light cream	Pinkish
Microscopic Morphology	*Cocci* Single	Rod Single	Rod Chain	*Cocci* Cluster
Morphological Characteristics				
Shape	Circular	Circular	Circular	Circular
Arrangement	*Cocci*	*Bacilli*	*Diplobacilli*	Cluster
Gram Reaction	+	+	+	+
Motility	Non-motile	motile	motile	Non-motile
Biochemical Test				
Catalase	+	+	+	+
Indole	-	+	-	-
Coagulase	-	+	+	+
MSAB	-	-	-	-
SCA	ND	ND	+	ND
Sugar Fermentation Test				
TSIA	+	+	+	+
Probably identify	*Micrococcus Species*	*Bacilli Species*	*Bacilli Species*	*Staphylococcus Species*

4.2 DISCUSSION

From the final result obtained from this research. Table1 shows the total number of viable bacteria counts derive from dilution 1 and 3 from different samples.

Note that more organisms was spotted in dilution 1 than in 3.

While table 2: shows the total numbers of bacterial isolates. It indicated the cultural, morphological and biochemical characteristics of bacteria isolated. The probable identity of the bacteria isolate where identified using the above method of classification.

***Micrococcus species*:** it belongs to the members of the family *Micrococcaceae*, are usually regarded as contaminants from skin and mucous membranes.Nevertheless they have been documented to be causative organisms in cases of *bacteremia, pneumonia* e.t.c. this bacterium can be transmitted due to poor hand washing practices. *Micrococcusspecies* can cause septic shock in immune compromised people.

***Bacillusspecies*:** it belongs to the phylum *bacillota*, with 266 named species. *Bacillusspecies* can be either obligate aerobes which are dependent on oxygen, or facultative anaerobes which can survive in the absence of oxygen.This species is generally transmitted through the consumption of food contaminated by it through the entry of soil, air, or water.

Staphylococcus species:they are topical gram-positive bacteria forming regular clusters of *cocci*. *Staphylococcus* is widespread in nature although they are mainly found on the skin, skin glands and mucous membranes of mammals and birds, but can cause infection under certain circumstances

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

In conclusion, results obtained from this study have shown that there is presence of bacteria contaminants spread through air borne in market areas may contribute considerably to the microbial burden of Garri sold in Uchi and Jattu markets Auchi, Edo State.

This is likely to be associated with poor post processing, handling practices such as spreading on the floor, mat and sometimes on high density polyethene, after frying to allow it to cool before sieving into finer gains and the open display in bowls and basins in the market measurement and aids of bare hands, coughing and sneezing while selling and the use of non-microbiologically determined hessian bags for packaging it.

5.2 RECOMMENDATION

As a result of the fact that, the focus on Garri should not only be on surface cleaning and hygienic handling during processing, but also on reducing or eliminating microbial air contamination during marketing and packaging of

products for sale and distribution, as safety is of particular concern with ready to eat food products. Environmental characteristics of the region should be considered while addressing marketing strategies. The findings suggest that the current mode of sale of Garri in local markets may pose potential risks for public health especially for vulnerable people and thus, It is recommend that use of hygienic packaging by producers and retailers in Auchi in order to ensure food safety and consumer protection. Also the various biochemical tests for isolation,determination of microbes, should be carried out to ascertain which microbe is actually present in the study.

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