

**BACTERIOLOGICAL QUALITY OF READY-TO-EAT AFRICAN SALADS (ABACHA)
SOLD AROUND THE UNIVERSITY OF BENIN, UGBOWO CAMPUS AREA,**

EDO STATE

BY

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CERTIFICATION

This is to certify that this project was carried out by Emmanuella Onyekachukwu NWAKAIRE (Miss) with Matriculation number LSC1906927 in the Department of Microbiology, University of Benin, Benin City.

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APPROVAL

I certify that this report was accepted in partial fulfillment of the requirement for the award of the B.Sc. (Honours) Degree in Microbiology.

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Date

DEDICATION

I dedicate this work to God Almighty. I also dedicate this work to my family and everyone whose care, support and contribution helped make this a success.

ACKNOWLEDGEMENT

I want to first appreciate God for his mercies and grace that has brought me this far, for helping me realize many of my dreams within this great citadel of learning. To him be all the glory and adoration.

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ABSTRACT

African salad popularly called “Abacha, "Abacha Ncha" or "Abacha and Ugba” contains lots of fresh raw vegetables and other ingredients which can be consumed without cooking. It is widely accessed for it is composed of food ingredients known to be rich in protein, carbohydrate, vitamins and minerals. The aim of this study was to determine the bacteriological quality of ready-to-eat African salads sold around the University of Benin, Ugbowo campus area, Edo state. Ready-to-eat African salad samples were purchased with and without "ugba" condiment from sellers in the Ugbowo campus area. All samples were serially diluted and inoculated on Nutrient agar, Eosin Methylene blue (EMB) agar and Simmons citrate agar. Bacterial counts from this study ranged from 5.15 ± 0.50 to $9.75 \pm 1.06 \times 10^4$ cfu/g. Pure cultures were obtained and seven different isolates were identified using cultural, morphological and biochemical characteristics. The isolates include *Bacillus* sp., *Salmonella* so., *Staphylococcus aureus*, *Citrobacter* sp., *Streptococcus* sp., *Staphylococcus* sp. and *Micrococcus* sp. *Bacillus* sp. had the highest percentage frequency of 29.41% while *Staphylococcus* sp. had the lowest percentage frequency of 5.88%. The presence of *Salmonella* sp. in the ready to eat African salad samples indicates poor hygienic practice during the processing of these foods which can pose a great health risk to consumers.

CHAPTER ONE

1.0

INTRODUCTION

1.1 Background of study

African salad popularly called “Abacha, "Abacha Ncha" or "Abacha and Ugba” by Igbo tribe of Nigeria, is an exotic delicacy and a special salad recipe native to Nigeria. The name African salad is thought to have originated from the Igbo’s ideology that salad contains lots of fresh raw vegetables and some other ingredients consumed without further cooking, therefore it is a salad and of African origin. African salad is widely accessed for its composition of food ingredients known to be rich in protein, carbohydrate, vitamins, and minerals. It can be eaten on its own or in combination with other snacks like coconut, palm kernel and groundnut. Though it can be as filling as any other main course meal, African salad is usually eaten as an in-between meal or as a side dish to the various Nigerian rice recipes (Maky, 2013). African salad is also regarded as a special delicacy during traditional festivals.

The preparation of African salad takes great efforts and the ingredients needed to prepare it vary according to ones taste and availability. The key to making a good African salad is to make sure that all the ingredients are well incorporated. It can include ingredients such as Ugba (*Pentaclethra macrophylla*), palm oil, potash, onions, nutmeg, crayfish, salt, pepper, maggi, ogiri (*Ricinus communis*), garden egg, garden egg leaves, Utazi leaves (*Gongronema latifolium*), Okazi (Ukazi) leaves (*Gnetum africana*), Ozeza (Uzeza) leaves (*Piper guineense*), kpomo (cow skin), meat and stockfish/fish (Miriam and Anthonio, 2011;Osewa, 2013). These ingredients are mixed thoroughly with the shredded cassava (Abacha). The ingredient added is dependent on one’s choice, purchasing power and availability. African salad can be served with fried fish/meat over a cold drink (Palm wine, beer, stout or wine) (Miriam and Anthonio, 2011;Osewa, 2013).

Abacha is renowned as a very special dish to the Igbos. It is prepared with dried shredded cassava, utazi leaves, pepper, potash, red oil, ugba (ukpaka), crayfish and diced garden eggs. These ingredients used in preparing abacha makes up its rich nutritional value. Abacha has a rich content of minerals and nutrients which includes: Calcium, Potassium, Magnesium, Sodium, Vitamin C, Zinc, Iron, Vitamin A and Phosphorus. Cassava is equally a great source of thiamine, riboflavin, and niacin. It also has additional nutritional values of Protein and Crude Fibre, due to the presence of other ingredients often added to the food (Ross *et al.*, 2002).

The health benefits of African salad are many and vary depending on the ingredients used in its preparation. However, abacha foods do have some health benefits; This is a great gluten-free Nigerian food that is ideal for people who are allergic to gluten consumption. Garden egg leaves used to prepare salads are quite rich in ascorbic acid, calcium and antioxidants. Utazi leaves are good for improving digestive health. It helps form strong bones, also prevents bone damage and can improve circulation and transport of blood throughout all the vessels of the body. Abacha is often eaten with animal proteins such as fish, chicken or kpomo, which is very good for the body. It is also rich in potassium, 1 cup of cassava contains 558 mg of potassium, good for the heart and cells. African salad also helps strengthen the body's immune system to fight external infections (Ross *et al.*, 2002).

In developing countries, a large proportion of ready to eat foods are sold on the street (Mensah *et al.*, 2002). According to the Food and Agriculture Organization, 2.5 billion people worldwide eat street food every day. Increased reliance of street food has been identified as one of the characteristics of urban food distribution systems driven by changes in the urban way of life and poverty in developing countries. Street foods have already become a common feature of urban

life. The increasing poverty and time constraints to survive in developing countries indicate that the street food phenomenon will only increase (Hilda, 2002). With the increasing pace of globalization and tourism, the safety of street food has become one of the major concerns of public health, and a focus for governments and scientists to raise public awareness of. Street foods play significant nutritional role for consumers, particularly for middle and low-income sectors of the population, who depend on street foods for their main food intake (Mensah *et al.*, 2002). The contribution to the daily food intake of poor urban dwellers is scarcely quantified in energy and nutrients (Hilda, 2002). Street food operations sometimes involve the entire family in the procurement of raw materials, preparation and cooking of the meals. The role of women in the sector is significant, as they control a large share of market activity and commodity trading. Street food vendors benefit from a positive cash flow, often evade taxation, and can determine their own working hours (Mensah *et al.*, 2002).

1.2 Justification of study

Street food is also regarded as tasty, distinguishable by its local flavor and can be purchased on the sidewalk, without entry into a building (Lues *et al.*, 2006). Most of these foods are not protected against insects and dust which may harbor foodborne pathogens (Rane, 2011). Pathogens present in street vended foods come from different sources and practices such as improper food handling, use of contaminated water, unhygienic environments and improper storage. Improper food handling can lead to transfer of pathogens such as *Salmonella*, *E.coli* and *S. aureus* from the human body and environment into foods (Rane, 2011). The use of contaminated water has been associated with pathogens such as *E.coli*, Fecal *Streptococci*, *Salmonella* and *Vibrio cholera* while vegetables and spices have been associated with

introduction of spore formers such as Bacilli and Clostridium and pathogens like *Listeria monocytogenes*, *Shigella sp.* and *Salmonella sp.* (Rane, 2011). Also, improper storage temperature and reheating of food have been associated with production of heat stable toxins produced by pathogens like *Clostridium perfringens* and *Bacillus cereus* (Rane, 2011).

1.3 Aim and objectives

The aim of this study was to determine the bacteriological quality of Ready-to-eat African salads sold around the University of Benin, Ugbowo campus area, Edo state.

The specific objectives were to;

1. determine the total heterotrophic bacterial count of Ready-to-eat African salad samples
2. isolate bacteria from Ready-to-eat African salad samples
3. identify and characterize bacterial isolates from African salad samples
4. carry out an antibiotic susceptibility test on the bacterial isolates.

CHAPTER TWO

2.0

LITERATURE REVIEW

2.1 Cassava

Cassava (*Manihot esculenta Crantz*), a 1-2 m high woody shrub with an edible root is a perennial crop. It is made up of an aerial part (2-4 m) and an underground part (edible root) clustered around the lowermost part of the plant and extends about 60 cm on all sides (Pandey *et al.*, 2000). A single root may weigh as much as four kilogram under convenient conditions. The number of roots per plant at harvest varies from 2 to 7, each averaging 27.7 to 43.3 cm long and from 4.5 to 7.4 cm in diameter. A central vascular core, the cortex (flesh) and the phelloderm (peels) makes up a mature root. Cassava peels account for 10–12% of the total dry matter of the root and are 1–4 mm thick. Cassava, one of the most useful tropical crops widely exploited as a cheap energy source in Africa, Asia, South America and India was however castigated as an inferior food crop, poor people's crop and a dangerous crop because of major limitations like low protein content, short postharvest shelf life and the presence of toxic cyanogenic glucosides. (Pandey *et al.*, 2000)

2.1.1 Origin of cassava

In tropical Africa, cassava has assumed the status of a security and industrial crop, alleviating the food crisis in many war-torn and drought ravaged parts of Africa because it can be cultivated throughout the year without regard to the seasons. It produces high yield and grows with limited water (Oyewole, 2002). Cassava was reported to have originated in Venezuela and South America during 2700B.C. However, it was Phillips (1982) introduced to Nigeria and other parts

of West Africa by the Portuguese. It was estimated by FAO that 37% of the 13.4 million ton world production was produced by African countries. The main cassava producing countries of Africa include Nigeria, Benin, Kenya, Zambia, Tanzania, Uganda, Ghana, Zimbabwe, Democratic Republic of Congo and Mozambique (Nweke *et al.*, 2002). In 1996, Soccol reported Africa as the largest producer with about 53% of the world's production. Although, it was cultivated in about 88 countries, only 5 countries accounted for 67% of the production and these include Nigeria, Brazil, Thailand, Zaire and Indonesia. Purseglove (1968) reported that cassava was known around the North of the River Niger in 1914 but it has now become one of the most important staple food crops of the inhabitants in Nigeria and known by different names among the ethnic groups of the country. The Yoruba call it '*gbaguda*' or '*ege*', the Hausa, '*rogo*', '*karaza*' or '*doyar*' '*kudu*', the Ibo, '*akpu*', '*abacha*' or '*jigbo*', the Benin, '*igari*', the Efik, '*iwa*' '*unene*' while the Urhobo refer to it as '*imidaka*' (Etejere and Bhat, 1985)

2.1.2 Importance and Consumption of Cassava

Cassava plays a major role in efforts to alleviate the African food crisis because of its efficient production of food energy, year-round availability, tolerance to extreme stress conditions, and suitability to present farming and food systems in Africa. Much recognition has been dwelled on the importance of cassava and among reported ones are its use as source of income and raw material in the manufacturing of processed food, animal feed as well as industrial products (Beléia *et al.*, 2004). Wider utilization of cassava products can be a catalyst for rural industrial development and raise the income for producers, processors and traders, contribute to the food security status of its producing and consuming households as well as its high efficiency in converting solar energy to starch. Total world cassava use was projected to increase from 172.7 million ton to 275 million ton over a period of 27 years (1993-2020), using the International

Food Policy Research Institute baseline data whereas, a higher prediction of demand and production growth puts the 2020 production at 291 million tons (Scott *et al.*, 2000). In both projections, cassava use in Africa is equivalent to 62% of total world production with an average of about 102 kg/person/year or 220 kcal/person/day and Nigeria is the current leading producing country (FAO, 2008). Almost all the cassava produced globally is used for human consumption, either in natural form as flour or in fermented forms and other products with only 5% being used industrially (FAO, 2002; Ajao and Adegun, 2009).

2.1.3 Shelf life of cassava

Other tuber crops such as yam and sweet potato are not as perishable as cassava roots (Poulter, 1995). Physiological deterioration of Cassava (primary or secondary) occurs 2-3 days after harvesting, followed by microbial deterioration 3-5 days thereafter (Akingbala *et al.*, 2005). Primary deterioration is an endogenous physiological process called vascular streaking brought about by damage to the roots during postharvest handling which results in a fine blue-black or brown discoloration. This usually occurs when phenolic compounds present in the tubers are converted to coloured compounds called quinines and the process is catalyzed by the enzyme, polyphenol oxidase which acts on the phenolic compounds in the presence of oxygen. Furthermore, dehydration caused by the physical damage to the tubers worsens the conditions thus, making the tissues become portals of entry for pathogens leading to the secondary deterioration by microorganisms. The microbial spoilage involves rotting, softening or fermentation of the tissue by microorganisms (Uritani *et al.*, 1984).

2.1.4 Cassava varieties

There are many natural cassava varieties (cultivars) and are classified according to morphological traits as well as taste, cyanide content, average yield, disease performance and pubescence (MIC, 2007; Gbadegesin *et al.*, 2013). However, in recent times, a number of regional programs have been initiated to breed improved varieties of cassava to increase yield and resistance to diseases. Various studies have shown that the physicochemical, functional and other quality characteristics of cassava products are significantly affected by varietal chemical composition differences such as dry matter, starch content and quality. Even though more than 5,000 cassava cultivars are recognized globally (Gade, 2003; IFAD/FAO, 2005), several improved cassava varieties have been recommended and released in Nigeria (IITA, 2004).

Cassava varieties are the types of cassava cultivar planted in Nigeria. The use of good cassava varieties can enhance cassava farming business. Some of the most popular cassava varieties in Nigeria include the following; TME 419, Sunshine, Game Changer, Hope, Obasanjo – 2, Baba – 70, Poundable (IITA, 2004).

2.1.4.1 TME 419

TNE 419 is one of the most prolific cassava varieties in Nigeria. It has a high starch content and suitable for the production of cassava flour, starch, glucose and ethanol. TME 419 cassava variety can yield over 45 tons per hectare if good agronomic practices are implemented. TME 419 is one of the most planted varieties used for cassava farming in Nigeria (IITA, 2004).

2.1.4.2 Sunshine

Sunshine is also one of the most planted cassava varieties in Nigeria. This cassava variety is suitable for the production of fufu and garri. Sunshine cassava variety can yield above 30 tons of fresh cassava tubers per hectare. Game Changer Game Changer is a cassava variety developed by the International Institute of Tropical Agriculture (IITA) and the National Root Crops Research Institute (NRCRI). It is a prolific variety as it can yield over 35 tons per hectare if well managed. Game Changer can be used for the production of starch and ethanol (IITA, 2004).

2.1.4.3 Hope

Hope is also a cassava variety developed by the International Institute of Tropical Agriculture (IITA) and the National Root Crops Research Institute (NRCRI). It has a high yield. It can yield over 30 tons per hectare with good agricultural practices. Hope can be used for the production of starch and ethanol (IITA, 2004).

2.1.4.4 Obasanjo-2

Obasanjo-2 is also a cassava variety developed by the International Institute of Tropical Agriculture (IITA) and the National Root Crops Research Institute (NRCRI). It can yield up to 40 tons and more if irrigation is used. Obasanjo-2 can be used for the production of garri and fufu. Baba 70 Baba-70 is also a cassava variety developed by the International Institute of Tropical Agriculture (IITA) and the National Root Crops Research Institute (NRCRI). It has a high starch content and suitable for the production of cassava flour, starch, glucose and ethanol. Baba 70 cassava variety can yield over 30 tons per hectare if good agronomic practices are implemented (IITA, 2004).

2.1.4.5 Poundable

Poundable is known for its high content of starch. It is widely cultivated by a lot of farmers in Nigeria. It can be used for the production of cassava flour, starch, glucose and ethanol. It can also be used to produce fufu and garri. Poundable cassava variety can yield over 30 tons per hectare if good agronomic practices are implemented. Other cassava varieties planted in Nigeria include the following: Dixon Fine Face Farmer's Pride MS 90257 TMS 91934 TMS 81/00110 TMS 82/00661 TMS 30001 TMS 30555 TMS 30572 Nwugo Nwaiwa Ekpe Okotorowa among the most commonly grown are TME 30572, 4(2)1425, 92/0326 and NR8082. The major genetic factor that determines quality of roots is dry matter content and more recently, 42 new improved genotypes have been made available to farmers with qualities like multiple resistance/tolerance to cassava mosaic disease and other major problems of cassava, bacterial blight disease, anthracnose, green mite, and mealybug (IITA, 2004).

2.1.5 Nutritional composition of cassava

Cassava roots, though deficient in protein (less than 1.5% of fresh weight), are rich in carbohydrates (31% of fresh weight) with most of it being present as starch hence, its utilization by most people in tropics as sources of carbohydrate (Blagbrough *et al.*, 2010). The edible starchy flesh comprises some 80- 90% total weight of the root as water, forming the major components and it is between the range of 60.3 - 87.1% (Padonou *et al.*, 2005; Zvinavashe *et al.*, 2011; Harris and Koomson, 2011). Water is an important parameter in the storage of cassava with low levels giving desirable and relatively longer shelf life (Padonou *et al.*, 2010; Harris and Koomson, 2011).

Cassava contains about 1-2% protein which makes it a predominantly starchy food (Charles *et al.*, 2005). The protein content is low (1% - 3%) on a dry matter basis and between 0.4 and 1.5% per 100 g fresh weight. About 50% of the crude protein in the roots consists of whole protein and the other 50% in the form of free amino acids (primarily glutamic and aspartic acids) and non-protein components such as nitrite, nitrate and cyanogenic compounds (Zvinavashe *et al.*, 2011). The root also has high content of dietary fibre, magnesium, sodium, riboflavin, nicotinic acid, thiamine and citrate but was reported to be low in iron and vitamin A. Cassava tuber has been criticized for its low and poor protein content, it produces more weight of carbohydrate per unit area than other staple food crops under comparable agro-climatic conditions thus, being an energy-dense food and therefore ranked high for its energy value of 250 x 10³ cal/ha/day as compared to 176 x 10³ cal/ha/day for rice, 110 x 10³ cal/ha/day for wheat, 200 x 10³ cal/ha/day for maize, and 114 x 10³ cal/ha/day for sorghum (Jisha *et al.*, 2010). Montagnac (2009) and Zvinavashe *et al.* (2011) reported that the root is a physiological energy reserve with high carbohydrate content, which ranged from 32% to 35% on a fresh weight (FW) basis, and from 80% to 90% on a dry matter (DM) basis. Raw cassava root however, has more carbohydrate than potatoes and less carbohydrate than wheat, rice, yellow corn, and sorghum on a 100 g basis (Montagnac, 2009). The lipid content in cassava roots ranges from 0.1% to 0.3% on a fresh weight basis with values ranging from 0.1% to 0.4% and 0.65% on a dry weight basis (Charles *et al.*, 2005; Padonou *et al.*, 2005). It has been found that cassava lipid content is low compared to that of maize and sorghum, but higher than potato and comparable to rice. The lipids are either non-polar (45%) or glycolipids (52%) according to Hudson and Ogunsua (1974), mainly galactose-diglyceride (Gil and Buitrago, 2002).

2.1.6 Anti-nutritional factors of cassava

Cassava has significant deficiencies that limit its usefulness as a food source, including the presence of cyanogenic glycosides; linamarin (2- β Dglucopyranosyloxy-2-methylpropanenitrile) and lotaustralin [(2R)-2- β Dglucopyranosyloxy-2-methylbutyronitrile] are derived from valine and isoleucine, respectively (Peifan *et al.*, 2004). The ratio of linamarin/lotuastralin in leaves and roots was reported by Nartey (1978) and Liang Cheng *et al.* (1995) while less than 83% linamarin has been reported by other researchers (Kimaryo *et al.*, 2000). Linamarin is a β -glucoside of cydrarrn and ethyl-methylketone-cyanhydrin stored in the vacuoles of cassava cells and their β -bonds can only be broken under pressure, high temperature and the use of mineral acids. Linamarin has a bitter taste, making cyanide-rich cassava (>100 ppm) taste bitter, thus being called bitter cassava. Cyanogen is present in concentrations varying from 300 to 500 ppm depending on the type of cassava. Therefore, bitter varieties, which contain higher amounts of cyanogenic glycosides, must be processed to remove toxic compounds before consumption, while sweet varieties (about 100 ppm) have low levels and Can be eaten fresh. Despite this, those who use cassava as a staple food mainly grow bitter varieties due to their higher yields, greater resistance to insects and pests, and therefore rely on processing methods for detoxification. Cardoso *et al.* (2005) estimated total cyanide content to range from 1 to 1,550 ppm.

Hydrocyanic concentrations ranging from 15 to 400 mg/kg fresh weight in cassava varieties have been reported, values more frequently range from 15 to 150 mg/kg although the minimum tolerance level is the recommended Nigerian standard is 50 mg. /Kg. (SON, 1985). Variations may be due to different rates of biosynthesis, degradation, or transport. Environmental factors, cultivar and growing conditions were also recorded. Cassava plants have endogenous

linamarase (β -glucosidase), an enzyme that can easily hydrolyze linamarin, located in the cell wall. Crushing or cutting the roots, due to damage to the cell structure, allows the release of endogenous linamarase capable of hydrolyzing linamarin to glucose and acetone cyanohydrin, under optimal conditions at 25°C and at a pH of 5, 5 to 6.0. As reported by Mkpogon *et al.* (1989) as well as Ikediobi and Onyike (1982), endogenous linamarase content cannot allow complete degradation of linamarin. It has been shown that cyanogenic toxicity can be reduced by introducing exogenous linamarase during fermentation. Authors have proposed inoculating linamarase-producing microorganisms into fermented cassava. This reduces cyanogen content because microorganisms produce linamarase that breaks down linamarin. Fermenting cassava with water is the simplest method to reduce cyanide content, as water will facilitate cell swelling and allow linamarase to come into contact with linamarin, leading to hydrolysis (Bradbury, 2006; Cumbana *et al.*, 2007; Bradbury and Denton, 2010).

Uyoh *et al.* (2009) also observed that the use of unchanged water during fermentation significantly reduced cyanide content. The reduction in cyanide content of fermented cassava, reported by Westby and Choo (1994), ranged from 65 to 110 mgHCN/kg over a period of 12 to 96 h, while Onyesom *et al.* (2008) placed the range between 7.02 and 2.70 mgHCN/100 g fresh weight of cassava over a period of 24 to 96 h. Although the majority of these toxic components have been removed during cassava processing, a certain amount remains, depending on the process used according to Nartey (1981) as well as Nambisan and Sundaresan (1985). Consuming cassava that still contains residual cyanogenic compounds can lead to chronic diseases such as goiter, cretinism, local ataxia neuropathy, iodine deficiency, cell destruction and tropical diabetes. Because cassava has absolutely no protein and is severely lacking in vitamins and minerals, consuming it alone can cause malnutrition (Bradbury and Denton, 2010).

2.2 Cassava fermentation

Fermentation of cassava was reported to be the most important and widely used means of processing cassava. As of date, the ancient traditional processing of cassava is still being used and this practice is however, afflicted with so many problems because it depends on chance inoculation from the environment. Thus, the fermentation period is rather slower, with inconsistent quality of the products from one processor to the other as well as from one production batch to the other even by the same processor, and from one season to the other. Improvements in cassava processing, which have been employed over the years has helped to reduce the duration of processing to economically viable limits, maximise the detoxification process and improve the physical and nutritional qualities of the products. While the methods of fermentation vary from one locality to another (grated root fermentation, underwater/soaking fermentation, and mold fermentation), cassava fermentation in sub-Saharan Africa has been categorized either as solid state or submerged (Nweke *et al.*, 2002).

Cassava roots are bulky with about 70% moisture content, and therefore transportation of the tubers to urban markets is difficult and expensive. Moreso, the raw roots and uncooked leaves are not palatable and they contain varying amounts of cyanide which is toxic to humans and animals. Therefore, cassava must be processed into various forms in order to increase the shelf life, facilitate transportation and marketing, reduce cyanide content and improve palatability. Traditionally, cassava roots are processed by various methods into numerous products and utilised in various ways according to local customs and preferences. In some countries, the leaves are consumed as vegetables, and many traditional foods are processed from cassava roots and leaves. The nutritional status of cassava can also be improved through fortification with other protein-rich crops. Processing reduces food losses and stabilizes seasonal

fluctuations in the supply of the crop. Various traditional processing methods are known which include boiling, smoking, drying and fermentation (Nweke *et al.*, 2002).

2.2.1 Submerged Fermentation

Submerged fermentation is the cultivation of microorganisms in liquid nutrient medium in which the bioactive compounds are secreted into the fermentation medium (Renge *et al.*, 2012; Subramaniyam and Vimala, 2012). Submerged fermentation of cassava involves the soaking of cassava roots under water for 3 -5 days, causing the root to soften and swell, thus having a combined effect of enabling linamarase and linamarin to mix as well as leaching of cyanogens. The softened roots can be easily broken into pieces by hand and then passed through a sieve to remove the fibre, leaving a smooth paste. Initially, a mixed microflora was reported to be involved, but it was later dominated by lactic acid bacteria. The size to which the roots were cut prior to soaking was also found to affect the rate of fermentation and the quality of product. Different groups of Lactic acid bacteria isolated from the submerged fermentation of cassava includes *Lactobacillus cellobiosus*, *Lactobacillus bulgaricus*, *Lactobacillus brevis*, *Lactobacillus coprophilus*, *Lactobacillus plantarum* and *Leuconostoc mesenteroides* with *Lactobacillus plantarum* being predominant during the last 36 hours (Achi and Akubor, 2000; Obilie *et al.*, 2004; Kostinek *et al.*, 2005).

2.2.2 Microbiology of cassava fermentation

Starch is a complex carbohydrate that can be degraded either by microorganisms that produce α -amylase or an inducible/constitutive amylase to produce simple sugars which can then be readily metabolised by many microorganisms. The indigenous natural fermentation has been

reported to involve mixed colony of microorganisms such as molds, bacteria and yeasts (Kobawila *et al.*, 2005; Ekundayo and Okoroafor, 2012). Even though, microbial size in food is usually small, their influence on the nature of the food, especially in terms of flavour, and other organoleptic properties, is profound (Okafor, 2009). Fermentation products are based on the microorganisms involved in the fermentation and the type of bacterial flora developed in each fermented food varies based on water activity, pH, salt concentration, temperature and substrate composition. These microorganisms are harmless to the consumer and produces enzymes such as proteases, amylases and lipases that hydrolyze food complexes into simple non-toxic products with desirable texture and aroma that makes them palatable for consumption (Blandino *et al.*, 2003).

Numerous authors have linked a wide spectrum of microorganisms to cassava fermentation and these includes *Bacillus*, *Leuconostoc*, *Klebsiella*, *Corynebacterium*, *Lactobacillus*, *Aspergillus*, *Candida*, *Geotrichum*, *Streptococcus*, *Enterococcus*, *Aerococcus* and *Pediococcus* species (Holzapfel, 2002; Blandino *et al.*, 2003). Furthermore, yeasts and molds such as *Saccharomyces*, *Candida*, *Kluyveromyces*, *Aspergillus*, *Rhizopus*, *Mucor*, *Penicillium* and *Debaryomyces* were also reported (Wouters *et al.*, 2002; Omemu *et al.*, 2007). Rapid growing Lactic Acid Bacteria (LAB) are the most common prominent microorganisms for fermentation and preservation of foods. Their importance was known to be associated mainly with their safe metabolic activity while growing in foods, utilising available sugar for the production of organic acids and other metabolites. Their common occurrence in foods, coupled with their long-lived use contributed to their natural acceptance as GRAS (Generally Recognised as Safe) for human consumption. *Lactobacillus plantarum* has been shown to be the predominant LAB specie in sour cassava starch, even though, most species were found not to produce α -amylase and this

was quite surprising because cassava has about 84% of the carbohydrates in the form of starch (Ben Omar *et al.*, 2000; Lacerda *et al.*, 2005; Kostinek *et al.*, 2007). Sanni *et al.* (2002) stated that only a few amylolytic LAB have been isolated from starchy fermented foods in Africa but more studies over time, had led to the discovery of more (Diaz-Ruiz *et al.*, 2003; Putri *et al.*, 2011a, 2011b; Mukisa *et al.*, 2012) . In other cases, there are many kinds of fermented foods in which the dominating processes and end products are dependent on a mixture of endogenous enzymes and mixed microbial cultures which were earlier reported to have originated from the native microflora of the raw materials utilised in most of the traditional food fermentation processes according to Anthony and Chandra (1997).

Some of the inhibitory compounds formed during fermentation include organic acids (e.g. palmitic, pyruvic, lactic, acetic, propionic and butyric acids), alcohols (mainly ethanol) aldehydes and ketones (acetaldehyde, acetoin, 2-methyl butanol). These varieties of metabolites are antagonistic in action to competing bacteria. The inhibition has been attributed to the protonated form of the acids, which are uncharged and may therefore cross biological membranes, thus inhibiting growth due to lowered pH of the cytoplasm and/or accumulation of anions inside the cell. In other words, they interfere with the maintenance of cell membrane potential, inhibiting active transport and a variety of metabolic functions as well as reducing intracellular pH (Ross *et al.*, 2002).

2.3 Cassava products

Some products of cassava in Nigeria include ‘gari’, ‘abacha’, ‘lafun’, ‘usi’, ‘fufu’, ‘tapioca cakes’.

2.3.1 'Fufu'

Fufu is traditionally produced and marketed as a fermented wet, pasty food product, which is also made into porridge in boiling water before consumption. Mostly consumed in the Eastern and Western parts of Nigeria as well as some other parts of West and Central Africa, it is known as 'chikwuangue' or 'chikwange' in Zaire; 'fufu' or 'foo-foo' in Southern Nigeria and 'akpu' in some parts of Eastern Nigeria (Longe, 1990). Traditional fufu fermentation involves peeling and washing cassava roots that are manually cut into different sizes by using a hand knife and soaking in earthen pots or drums of water for 3 to 5 days to undergo lactic acid fermentation. Reports indicated that during soaking, the pH value decreases, the root softens and this facilitates the reduction in potentially toxic cyanogenic compounds (Uyoh, *et al.*, 2009). The soft roots are broken with clean hands and the fibres removed by sieving which is done by adding water to the retted mass on a sieve. The starch suspension is allowed to sediment for about 24 hours after which the water is decanted. Fine, clean starch is further dewatered by putting in raffia or cotton bags and pressed with heavy stones overnight. To prepare for consumption, a quantity of the slurry containing about 25% of fufu paste in water was boiled in an open pan. After continuous stirring using a wooden rod, strong dough was formed. This method is commonly reported among the Yoruba tribe. The Igbo and Efik tribes of Eastern Nigeria who refer to it as nni akpu and udep utim respectively, rolls the starch into large balls, wrap it with wilted plantain leaves and partially steam cook. The balls are removed and pounded in a wooden mortar to give a fine, smooth and soft mash. It is further rolled into small balls, wrapped in leaves and thoroughly steamed, after which the balls are finally pound together. The cooked fufu is usually eaten warm with fish, meat, vegetable stew or soup.

Unlike other fermented cassava products, it has a very strong odour and considered by consumers to be of good quality when it has a smooth texture, characteristic aroma and creamy white, grey or yellow colour. Variability in the quality of fufu could be as a result of chance inoculation involving varieties of microorganisms, little or no control over the process, roots cut size, difference in dry matter content, root quality and fermentation water (Oyewole and Sanni, 1995; (Uyoh, *et al.*, 2009)).

2.3.2. 'Usi'

'Usi' is one of the major native foods of the Itsekiri and Urhobo in Southern Nigeria, who also refer to it as edible starch. At household level, different techniques are used to obtain the fermented starch. It may be precipitated from the solution pressed out of the grated cassava roots or from grated cassava that is soaked directly in water (Uyoh, *et al.*, 2009). The starch is produced either in a wet form or more commonly, dried. The cassava roots are peeled, washed and grated. The grated pulp is steeped for 2-3 days in a large quantity of water. The mixture is stirred and filtered through a piece of cloth sieve. The filtrate stands overnight and the supernatant is then decanted. The fine starch paste is collected and put in a wide metal pan that is already smeared with red palm oil. Water is added and then stirred with the hand to dissolve completely. The pot is put on fire and the solution constantly stirred with a wooden rod until it is converted to a very sticky, light yellow mass. This is eaten with any oil or soup (Uyoh, *et al.*, 2009).

2.3.3 ‘Abacha’

The ‘Abacha’ food, which is popularly regarded as African Salad in English, is a very aromatic delicacy and quite nutritious to the enzymes of the stomach. This native dish is peculiar to Igbos, which are predominantly found in the South East part of Nigeria. Furthermore, the food is said to have originated from the South Easterners and “Abacha” is one of the Igbo’s native words. The African salad dish, is also called ‘*Abacha Ncha*’, and ‘*Abacha*’ and ‘*Ugba*’, by the Igbo people of Nigeria. It is a favourite choice for most Igbo traditional outings, and it is often served to guests. Abacha can be relished as an appetizer, dessert, snack, or as a normal meal, any day, any time. Without any iota of doubt, it is truly one healthy and nutritious meal. The ingredients required for preparation of abacha include; Dried shredded cassava, ugba or ukpaka, palm oil, powdered potash, fish (spiced cooked), ponmo (cooked and sliced), onion (sliced), garden eggs (diced), garden egg leaves (chopped), salt and dry pepper, crayfish, seasoning cubes, calabash nutmeg, ogiri, fresh utazi leaves and boiling water (Uyoh, *et al.*, 2009).

3.3.3.1 Preparation/Production of wet abacha from slices dried cassava chips

Freshly harvested cassava roots from different varieties are manually peeled with stainless steel knife, washed and cut into chunks (7.0 – 8.0 cm length). They were washed and spread on a flat basket made from palm frond woven material and dried for 5 days.

The dried chips are hydrated in potable water to attain a high moisture content (about 65%) in 7 h and then boiled in water for 75 min. The cooked samples are cooled and sliced thinly (0.50 – 0.80 mm thick) with a sharp stainless steel knife. The sliced samples were soaked in water for 16 h and the water is routinely changed after 4 h. After soaking, the slices are thoroughly washed to

obtain fresh wet 'abacha' slices. Thereafter the fresh wet 'abacha' slice are then oven dried at 65°C, 6 h to obtain dry 'abacha' slices (Uyoh, *et al.*, 2009).

3.3.3.2 Preparation of abacha from fresh cassava roots

Freshly harvested cassava roots are manually peeled with stainless steel knife, washed, cut into chunks (7.0 – 8.0 cm length) and boiled in water for 20 min. The cooked samples are cooled, thinly sliced (0.50 – 0.80 mm thick) and soaked for 16 h. During the soaking, the water is routinely changed after 4 h.. The slices are properly washed to obtain fresh wet 'abacha' slices. The fresh wet 'abacha' was also oven dried (65oC, 6 h) (Uyoh, *et al.*, 2009).

2.4 Nutritional Value Of Abacha

Abacha is renowned as a very special dish to the Igbos. It is prepared with dried shredded cassava, utazi leaves, pepper, potash, red oil, ugba (ukpaka), crayfish and diced garden eggs. These ingredients used in preparing abacha makes up its rich nutritional value. Abacha has a rich content of minerals and nutrients which includes: Calcium, Potassium, Magnesium, Sodium, Vitamin C, Zinc, Iron, Vitamin A and Phosphorus. Cassava is equally a great source of thiamine, riboflavin, and niacin. It also has additional nutritional values of Protein and Crude Fibre, due to the presence of other ingredients often added to the food (Ross *et al.*, 2002).

2.5 Health Benefits Of 'Abacha'

The health benefits of African salad are many and vary depending on the ingredients used in its preparation. However, abacha foods do have some health benefits; This is a great gluten-free

Nigerian food that is ideal for people who are allergic to gluten consumption. Garden egg leaves used to prepare salads are quite rich in ascorbic acid, calcium and antioxidants. Utazi leaves are good for improving digestive health. It helps form strong bones, also prevents bone damage and can improve circulation and transport of blood throughout all the vessels of the body. Abacha is often eaten with animal proteins such as fish, chicken or kpomo, which is very good for the body. It is also rich in potassium, 1 cup of cassava contains 558 mg of potassium, good for the heart and cells. African salad also helps strengthen the body's immune system to fight external infections (Ross *et al.*, 2002).

2.6 Street food/ready to eat foods

In developing countries, a large proportion of ready to eat foods are sold on the street (Mensah *et al.*, 2002). According to the Food and Agriculture Organization, 2.5 billion people worldwide eat street food every day. Increased reliance of street food has been identified as one of the characteristics of urban food distribution systems driven by changes in the urban way of life and poverty in developing countries. Street foods have already become a common feature of urban life. The increasing poverty and time constraints to survive in developing countries indicate that the street food phenomenon will only increase (Hilda, 2002). With the increasing pace of globalization and tourism, the safety of street food has become one of the major concerns of public health, and a focus for governments and scientists to raise public awareness of. Street foods play significant nutritional role for consumers, particularly for middle and low-income sectors of the population, who depend on street foods for their main food intake (Mensah *et al.*, 2002). The contribution to the daily food intake of poor urban dwellers is scarcely quantified in energy and nutrients (Hilda, 2002). Street food operations sometimes involve the entire family in

the procurement of raw materials, preparation and cooking of the meals. The role of women in the sector is significant, as they control a large share of market activity and commodity trading. Street food vendors benefit from a positive cash flow, often evade taxation, and can determine their own working hours (Mensah *et al.*, 2002).

2.6.1 Factors affecting food handling practice of food handlers

Age, marital status, service year, monthly income, food hygiene and safety training, attitude, knowledge and depth of knowledge were identified as factors affecting food safety practices according to Gizaw *et al.*, (2014). In other similar study marital status, monthly income, knowledge, presence of insects and rodents, existence of shower facility and separate dressing room were found to be significantly associated with food handling. According to a study by Legesse *et al.*, (2017) the following factors were associated with food safety practice; educational background, food safety training, food safety attitude, and practical three Compartments dishwashing system was. Moreover, Food handler whose age greater than 29-34 and ≥ 35 years respectively, having supervisor and medical check up, those who take training on food sanitation in the past were the identified significant factors associated with food handlers practice.

2.6.2 Microbial quality of street foods

A greater challenge to food safety is microbiological hazards because harmful microorganisms can either proliferate in the food or in the human body once ingested (Tent, 1999).

Shigella species was isolated 2.3% in Debre Markos university food handlers, (2.7%) in food handlers at Gondar University, (3.1%) at Gondar town, 0.4% from Hawassa and (3%) at Arba Minch University. 3.6% *Salmonella* species was isolated from food handlers in Debre

Markos university food handlers, (3.5%) in food handlers from Addis Ababa University, 6.9% among food handlers at Arba Minch University and Gondar University cafeteria (1.3%), 1.6% Bahir Dar town food handlers.

Shigella and *Salmonella* species have been isolated from 2.3% and 3.5% of food handlers (Teferi, 2020). A study conducted by Bereda *et al.* (2016) reported that out of 137 street vended food samples 72% were contaminated with pathogenic bacteria. The isolated bacteria were *E. coli*, *Staphylococcus aureus* and *Salmonella* sp. Another study by Amare *et al.* (2019) conducted on 72 street vended food samples revealed that 44 samples showed the presence of bacteria contaminants. Also previous studies have reported the prevalence of *Salmonella* and *Shigella* so in food handlers.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Sample collection

Ready-to-eat African salads ('Abacha') were purchased from four sellers located at the University of Benin Pedestrian bridge area and 3 other locations around the Ugbowo main campus area, Edo State, Nigeria. Two sets of samples were purchased from each seller; Ready-to-eat African salad with 'Ugba' condiment and Ready-to-eat African salad without 'Ugba' condiment. Each sample was purchased in triplicates. All samples were labelled accordingly and transported in sterile containers to the laboratory for analysis.

3.2 Sterilization of materials

All glass wares which include conical flask, test tube and Maccartney bottles were washed with detergent and distilled water. They were then wrapped with aluminium foil and sterilized in an oven for an hour at the temperature of 160°C.

3.3 PREPARATION OF CULTURE MEDIA

The media used in the study was prepared according to manufacturer's instructions employing standard laboratory practices.

3.3.1 Nutrient Agar 28g of nutrient powder was weighed and dissolved in 1 liter of distilled water in a conical flask corked with cotton wool and aluminum foil. The medium was then autoclaved at 121°C for 15 minutes. The medium was allowed to cool between 45°C to 50°C and then 20ml was poured aseptically into each of the petri dishes containing 0.1 of the aliquots.

3.3.2 Eosin Methylene Blue

37.5g of powdered EMB agar was dissolved in 1000ml of deionized water. It was mixed properly and autoclaved at 121⁰C for 15minutes. The medium was allowed to cool at 47⁰C and poured aseptically into petri dishes.

3.3.3 Simmons citrate Agar

24g of powdered Simmon citrate agar was dissolved in 1000 ml of deionized water. The medium was then autoclaved at 121⁰C for 15. The medium is set as a slope ensuring that the slant is over a butt about 3cm deep.

3.4 Enumeration and Isolation of bacteria

The total viable counts of the isolates were enumerated according to the method of Holt *et al.*, (1994). The total bacterial count at the dilution factor 10⁻³ was used to estimate the total viable count for the sample in colony forming units (cfu/g).

$$\text{Total bacteria count (cfu/g)} = \frac{\text{number of colonies}}{\text{Volume plated} \times \text{Dilution factor}}$$

3.4.1 SERIAL DILUTION

20 gram of the abacha samples was weighed and transferred aseptically into a test tube containing 9ml of sterile distilled water and shaken vigorously to dislodge the associated microorganisms. The suspension was subsequently serially diluted using ten-fold serial dilution up to 10⁻⁵.

3.4.2 POUR PLATE TECHNIQUE

0.1ml aliquot of dilution was placed in sterile petri dishes using the pour plate technique and 20ml of sterile cool molten agar was added. The dilution 10³ was selected for enumeration of

bacteria using nutrient agar. All plates are swirled to mix and the agar was allowed to solidify. Upon solidification, the plates were incubated in an inverted position at a temperature of 37°C for 24hrs.

3.4.3 SUBCULTURING OF BACTERIAL ISOLATE

A single isolated colony of the bacteria was picked up from a previous culture using a sterilized wire loop and was streaked on a fresh nutrient agar and EMB agar medium. The nutrient agar plates were incubated at 37 °C for 24 hrs. The purified and isolated bacterial strains were stored in a refrigerator.

3.5 CHARACTERIZATION AND IDENTIFICATION OF BACTERIAL ISOLATES

3.5.1 DETERMINATION OF CULTURAL CHARACTERISTICS

Cultural characteristics of the bacterial isolates were observed on nutrient agar plates. The cultural characteristics include; colony size, shape, surface appearance, opacity, texture, elevation and pigmentation. These were determined by visual observation.

3.5.2 MORPHOLOGICAL TESTS

3.5.2.1 GRAM STAINING TEST

The gram staining technique was used for differentiation between gram positive and gram negative bacterial strains according to Henraj *et al.*, (2013). A drop of sterile distilled water was placed on a neat and clean glass slide, and a single isolated colony of 24 hours old culture was mixed in it. The smear was made by spreading the culture. This smear was air dried and fixed by rapidly passing the slide three times over the flame. It was then flooded with crystal violet for 1minute and then washed off with distilled water. The gram's iodine solution was added to the

smear and the glass slide was left for 1 minute and then rinsed with distilled water. This step was followed by the application of decolorizing agent (ethanol) for 30 seconds. Decolorizing agent was immediately washed with distilled water and the smear was counter stained with safranin for 1 minute. The slide was washed with distilled water; air dried and was observed under the microscope at 100x, oil immersion objective.

3.6 BIOCHEMICAL TEST

3.6.1 OXIDASE TEST

Filter paper was soaked with 2 drops of freshly prepared oxidase reagent. Colony of the test organism was smeared on the filter paper using sterile wire loop. Positive oxidase was indicated by the production of a deep purple/blue color within 10 seconds (Cheesbrough, 2000).

3.6.2 INDOLE TEST

This was carried out according to the protocol outlined by Cheesbrough (2000). The test organism was inoculated into a Bijiou bottle containing 3 ml of sterile peptone water, incubated at 35-37°C for 48 hours. This was followed by the addition of 0.5 ml Kovac's reagent. Red color on the surface layer within 10 minutes indicated positive test for indole (Cheesbrough, 2000).

3.6.3 CATALASE TEST

1 ml of hydrogen peroxide solution was discharged into a clean glass slide and a sterile inoculating loop was used to collect the colonies of the test organism which were subsequently immersed in the hydrogen peroxide solution. A positive result was indicated by the production of gas bubbles, while its absence was regarded as negative.

3.6.4 CITRATE UTILIZATION

5ml of Sammon Citrate Broth was inoculated with the test organism. The broth was incubated at 37°C for 48hours. A positive reaction was indicated by a change in the color of the medium from green to blue color. Negative tubes were observed daily for 4days to detect any delayed reaction.

3.7 SUGAR FERMENTATION TEST

Many bacteria species can be differentiated on the basis of the sugars they utilize and ferment. The fermentation medium can be prepared by the addition of 0.1g of peptone, 0.1g of sodium chloride and 0.1g of fermentable sugar (glucose, mannitol, lactose, sucrose and mannose) in 10ml of distilled water. About 1ml of phenol red indicator was also added to the tubes. The Bijou bottles containing the sugar solution were inoculated with the test bacterial isolates and incubated at 37 °C for 24 to 48hours. After incubation, a change of color from red to yellow indicates acid production and the presence of gas in the inverted Durham tubes was indicative of gas production (O'Hara *et al.*, 1993).

3.8 ANTIBIOTIC SUSCEPTIBILITY TEST

Kirby Bauer agar disc diffusion technique was used for this test as described by Aromolaran and Badejo (2014) and Akinyemiet *al.* (2005). Pure colonies of bacterial isolates were streaked on sterile Muller Hinton agar (MHA) plates and incubated at 37 °Cfor 24 h. The bacterial cells were harvested into sterile normal saline solution and standardized using 0.5 McFarland standards. The cultured cells were introduced on the surface of sterile MHA using sterile swab sticks and multi-disc antibiotics were placed on the culture media aseptically and incubated at 37°C for 24hrs. The diameter of the zones of inhibition around each disc was measured after the

incubation period and recorded. Interpretation of results were carried out by adopting the breakpoints of Clinical and Laboratory Standard Institute.

CHAPTER FOUR

RESULTS

Table 1 shows the total heterotrophic bacterial count (cfu/g) of Ready to eat African salads around the University of Benin, Ugbowo campus area. The total bacterial count range from 5.15 ± 0.50 to $9.75 \pm 1.06 \times 10^4$ cfu/g.

Table 2 shows the cultural, morphological and biochemical characteristics of bacteria isolated from Ready-to-eat African salad. The bacteria include *Bacillus* sp., *Salmonella* sp., *Staphylococcus aureus*, *Citrobacter* sp., *Streptococcus* sp., *Staphylococcus* sp. and *Micrococcus* sp.

Table 3 shows the frequency of occurrence(%) of bacterial isolates in this study.

Table 4 shows the Antibiotic susceptibility pattern of the bacterial isolates obtained from the samples.

TABLE 1: Total heterotrophic bacterial count obtained from Ready-to-eat African salads sold around the University of Benin, Ugbowo Campus Area (10^4)

Sample code	Total bacteria count(cfu/g)
A	9.75 ± 1.06
B	8.85 ± 0.50
C	5.75 ± 0.32
D	5.90 ± 1.27
E	5.15 ± 0.50
F	5.60 ± 1.27
G	5.80 ± 0.57
H	5.65 ± 0.64

KEY

- a - Abacha sample with Ugba condiment, Pedestrian Bridge.
- b - Abacha sample without Ugba condiment, Pedestrian Bridge.
- c - Abacha sample with Ugba condiment from Location 2
- d - Abacha sample without Ugba condiment from Location 2
- e- Abacha sample with Ugba condiment from Location 3
- f - Abacha sample without Ugba condiment from Location 3
- g - Abacha sample with Ugba condiment from Location 4
- h - Abacha sample without Ugba condiment from Location 4

Table 2: Cultural, Morphological and Biochemical characteristics of bacterial isolated from Ready-to-eat African salads.

Cultural characteristics						
Colour	cream	Cream	Cream	Cream	Cream	Cream
Shape	Rhizoid	Circular	Irregular	Round	Circular	Circular
Elevation	Flat	Raise	Flat	Raise	Raise	Raise
Margin	Filamentous	Entire	Undulate	Smooth	Entire	Entire
Size	Small	Large	Large	medium	Small	Large
Morphological characteristics						
KOH	-ve	-ve	+ve	-ve	+ve	-ve
Gram stain	+ve	+ve	-ve	+ve	-ve	+ve
Cell morphology	Rod	Cocci	Rod	Cocci	Rod	Cocci
Cell arrangement	Chain	cluster	Singly	Cluster	Cluster	Cluster
Biochemical characteristics						
Catalase	+	+	+	+	+	+
Indole	-	+	+	-	+	+
Oxidase	+	-	-	+	-	-
Voges-proskauer	Variable	Variable	Variable	+		Variable
Spore forming	-	-		-		-
Glucose	+	+	+	+	+	+
Lactose	+	-	+	+	+	-
Sucrose	-	+	+	+	+	+
Mannitol	+	-	+	+	+	-
H ₂ S production	-	+	-	-	-	+
Identity	<i>Citrobacter</i> sp.	<i>Micrococcus</i> sp.	<i>Salmonella</i>	<i>Staphylococcus</i> <i>aureus</i>	<i>Bacillus</i> sp.	<i>Staphylococcus</i> sp.

Table 3: Percentage frequency of occurrence of bacterial isolates

ORGANISM	FREQUENCY (%)
<i>Bacillus</i> sp.	29.41
<i>Salmonella</i> sp.	11.76
<i>Staphylococcus aureus</i>	11.76
<i>Citrobacter</i> sp.	11.76
<i>Streptococcus</i> sp.	11.76
<i>Staphylococcus</i> sp.	5.88
<i>Micrococcus</i> sp	17.65

Table 4: Antibiotic susceptibility pattern of bacterial isolates

Isolates	NO. of susceptible isolates(%)								
		CPX	AM	AU	CN	PEF	OFX	S	CH
<i>Bacillus</i> sp.	5(100)	0(0)	2(40)	0(0)	0(0)	0(0)	0(0)	5(100)	0(0)
<i>Salmonella</i> sp.	2(100)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
<i>S. aureus</i>	2(100)	0(0)	1(50)	0(0)	0(0)	0(0)	0(0)	1(50)	(0)
<i>Citrobacter</i> sp.	2(100)	0(0)	2(100)	0(0)	0(0)	0(0)	0(0)	1(50)	1(50)
<i>Streptococcus</i> sp.	2(100)	0(0)	2(100)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
<i>Staphylococcus</i> sp.	1(100)	0(0)	1(100)	0(0)	0(0)	0(0)	0(0)	(0)	(0)
<i>Micrococcus</i> sp	3(100)	0(0)	3(100)	0(0)	0(0)	0(0)	0(0)	3(100)	0(0)

KEY

CPX- Ciprofloxacin

AM- Ampicillin

AU- Augumentin

CN- Gentamicin

PEF- Perfloxacin

OFX- Tarivid

S- Streptomycin

CH- Chloraphenicol

CHAPTER FIVE

DISCUSSION

The result of the study shows that the mean total bacterial count ranged from $5.15 \pm 0.50 \times 10^4$ to $9.75 \pm 1.06 \times 10^4$ cfu/g. The mean total bacterial count obtained in this study was lower than that reported by Daniel *et al*, (2016) who recorded bacterial count of ranging from 10.40×10^3 to 113.00×10^3 and $2.7.00 \times 10^3$ to 200.00×10^3 in African salad sold around Oba market and New Benin markets of Benin City Nigeria.

Bacteria isolated in this study include species of the genus *Bacillus* , *Salmonella*, *Staphylococcus*, *Citrobacter*, *Streptococcus*, *Staphylococcus* and *Micrococcus*. Daniel *et al*, (2016) also isolated species of the genus *Salmonella* and *Staphylococcus* from African salad samples. The presence of *Salmonella* sp. in the ready to eat African salad samples may have resulted from poor hygiene during processing of the food or the used of feacally contaminated vegetables and water for processing. *Salmonella* sp. have been reported to be a major contaminates of raw vegetable from the field due to the use of manure as fertilization as well as contaminated water for irrigation of vegetable during cultivation. The fact that the bacteria is mostly present on uncooked and raw food items, also 10^2 presence in this food indicates a diarrheal risk for consumers of the contaminated salad. The presence of *Staphylococcus* sp. in this study maybe attributed to improper handling during slicing of the vegetables and processing of cassava.

Bacillus sp. *Salmonella* and *S. aureus* showed high susceptibility to Ciprofloxacin. Ampicillin, Gentamicin, and Perfloacin did not was not susceptible to antibiotic. All isolates in the study were highly sensitive to Ciprofloxacin 5 (100%). Ciprofloxacin was proved to be the most

effective against Gram positive and Gram negative isolates studied in this research. These findings also agree with the findings of Geoffrey *et al*, (2013) in which the isolates Gram positive and Gram negative isolates all showed 100% sensitivity towards Ciprofloxacin. This can be attributed to the broad spectrum of activity of this antibiotic (Arora, 2008).

CONCLUSION

The presence *Bacillus* sp. *Salmonella* sp. *S. aureus*, *Citrobacter* sp. *Streptococcus* sp. and *Staphylococcus* sp. isolated from ready to eat African salad samples indicated the use of poor hygienic procedures during the processing of these foods which can pose a great risk to consumers. Proper washing of vegetables, use of clean utensils and practice of good hygienic and sanitary conditions can reduce the contamination of Abacha. Also, education of food handlers and the general public on food safety measures, effective Hazard Analysis Critical Control Point application and Good Manufacturing Practices (GMP) implementation is imperative.

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