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# Microbial and Biochemical Qualities of Vendor Dispensed Food - African Salad *(Abacha)* From Five Sub-Urban Areas of Owerri, Imo State

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**Abstract:** *The biochemical and microbial qualities of vendor dispensed African salad (Abacha) from five sub-urban areas in Owerri, Imo state were studied. The methods adopted were standard physicochemical and microbiological methods such as gram staining, motility test, citrate utilization test, catalase test, indole test, coagulase test, oxidase test, methyl red test, vogues proskauer test. The result obtained showed that the potash concentration range from 350mg/l in Ihiagwa to 740mg/l in Eziobodo, biochemical electrolytes such as iron, copper ion ranged from 9.35mg/l in Umuchima to 18.80mg/l in Eziobodo and 1.39mg/l in Ihiagwa to 2.53mg/l in Umuchima respectively, pH value was also determined and the pH range from 4.5 in Umuchima to 5.8 in Nekede , the mean aerobic plate count range from 2.6 $X10^4$ in Nekede to 9.1 $X10^5$ cfu/ml in Ihiagwa, coliform count range from 1.6 $X10^4$ in Nekede to 1.5 $X10^6$ cfu/ml in Umuchima, faecal coliform count range 3.2 $X10^4$ in Nekede to 4.5 $X10^5$ cfu/ml in Obinze and fungal count range from 6.5 $X10^2$ in Umuchima to 1.7 $X10^3$cfu/ml in Ihiagwa. The predominant bacterial isolates from the African salad samples were Escherichia coli, Bacillus sp. and Staphylococcus sp. The fungal species isolated included Mucor sp and Aspergillus niger. The possible sources of the organisms were highlighted and associated health risk discussed. It is therefore been recommended that adequate public health action is needed to forestall the adverse consequences of vending unwholesome food.*

**Keywords**: Microbial, Biochemical, Vendor, Aspergillus, Bacillus, Potash

## Introduction

Food is one of the basic requirements for life and to perform the intended use food must be nutritionally complete and be free of any injurious substances. A lack or insufficiency of food; or consumption of food containing harmful substances may result in dietary illnesses i.e. pellagra, rickets, marasmus, kwashiorkor and keratosis or food borne diseases i.e. cholera, dysentery, hepatitis, salmonellosis and typhoid (Marriott, 1999, Shapton & Shapton, 1993) According to the Food and Agricultural Organization (1989) food should also contribute to enjoyment and is also important in many social events and celebrations. Hence the critical need for food quality and safety, especially in retail food establishments such as food kiosks. Changes in food may occur in this food kiosks resulting into proliferation of micro-organisms responsible for food borne diseases.

According to survey carried out in 2006 and 2007 by the department of public health of the Federal Ministry of Health, there are more than two million recorded cases of food borne diseases in the country with the number of death put at over five (5) hundred. Some of these cases could be linked to consumption of contaminated street vended foods. However, the largely unregulated nature of the trade and poor hygienic practices as well as, lack of running water, toilet, proper storage and waste disposal facilities at preparation and services points has resulted in poor unsanitary conditions exposure to potential contaminants and an increased risk to public health. Consequently, the street food safety has remained a major public health concern globally and more importantly, in Nigeria where the regulation of this critical sector is virtually non-existent or inadequate, making street foods hazardous source of nutrition

Ready-to-eat foods of which African salad (Abacha) is one of them have been described as vehicles for the dissemination of microbial diseases caused by food borne pathogens. Studies have shown that sources of contamination include poor environmental sanitation, poor personal hygiene of food handlers and poor preparation and storage. Most of the vendors have no formal education and therefore lack adequate knowledge on proper food handling and therefore play a role in transmission of pathogens.

## Aim of the Study

The aim of this study is to establish the microbiological and biochemical properties of ready-to-eat food (African salad), sold in five sub-urban areas in Owerri, Imo State.

## Methodology

### Study Area

### Ihiagwa and it's Environ

Ihiagwa is a West African community located in the government area of Owerri West in Imo State

Nigeria. It is located 12km South of the capital city of Owerri. Ihiagwa is located at latitude of 5N, longitude 7E, Altitude 156m. The township is composed of eight villages: Umuelem, Umuchima, Mboke, Nnkaramochie, Iriamogu, Aku/Umokwo, Ibuzo and Umuezeawula. The market in Ihiagwa is called "Nkwo ukwu". Ihiagwa people are Igbo speaking ethnic group in the South eastern part of Nigeria with a population of 10,000. The major occupation of the people are farming and trading.

**Nekede and It's Environ**

Nekede is a town in South Eastern Nigeria. It is located near the city of Owerri. This is an Igbo speaking town that is made up of three distinct villages, viz Umuoma, Umualum and Umudibia (Federal Polytechnic Nekede Host). This town also hosts the Imo State new Owerri capital. The Federal university of Technology Owerri is 20 minutes drive from Nekede. It lies on the junction of the Nworie River and the Otamiri River. The population of Nekede is fast developing into a city with increasing population due to the location of the institution (Federal Polytechnic Nekede)

**Sterilization of Materials**

All glass wares such as Petri dishes, bijou bottles and glass containers were washed drained and dried. They were wrapped with aluminum foil and sterilized in hot air oven at $160^0$c for one hour. After sterilization, they were allowed to cool to about $40^0$c before use. Wire loops and straight wire were sterilized by flaming the wires in a naked gas flame until red hot. Dipping spreader in absolute alcohol and passing the glass spreader across a burning flame and allowing the alcohol to burn itself off sterilized glass spreader. Aseptic working environment was achieved with use of spirit lamp flame.

**Collection of Samples**

A total of five African Salad (Abacha) samples from five different sub-areas in Owerri were collected and placed aseptically in sterile polythene containers and were labeled at the collection points. The samples were transported to the laboratory within two hours.

**Microbiological Analysis**

Conventional spread plate method was used to enumerate the microbial groups in the African salad ( abacha) sample ($10^{-2}$, $10^{-3}$, $10^{-4}$). One milliliter of each dilution of $10^{-2}$, $10^{-3}$and $10^{-4}$ were pipette and transferred into separate quadruplicate well labelled sterile petri dishes. The plates were inverted and incubated at $35^0$c for 24hours for nutrient agar plate for total viable counts, $37^0$c for 24-48 hours for McCartney agar plates for coliform/enterobacter iceae counts, $37^0$c for 24-48 hours Eosin methylene blue plates for fecal coliform counts and $25^0$c for Sabouraud Dextrose Agar plate. The cultured plates were examined after incubation and colonies per plates were counted and recorded for estimation of colony forming unit per grammes (cfu/g) of original sample.

**Purification of Isolates**

After incubation, colonies developing on plates were randomly picked; sub cultured until pure cultures were obtained on corresponding agar plates for purification and preserved on appropriate agar slants, kept in the refrigerator at $4^0$c - $8^0$c for further studies.

**Indentification Of Bacterial Isolates**

Discrete colonies were identified based on cultural characteristics (size, shape, colour, margin, surface texture and elevation). The isolates were subjected to morphological and biochemical test to ascertain their identity. The morphological and biochemical characteristics of the respective bacterial isolates were then compared with the recommendation in Bergey's manual of Determinative Bacteriology (1989).

**Gram Staining**

This method was carried out according to Isenberg, (1992). Gram staining is used to characterize bacteria as Gram-positive or Gram-negative, based on the chemical and physical properties of their cell wall.

**Biochemical Identification of the Bacterial Isolates: Citrate Utilization Test (Using Simmon Citrate Agar)**

This method was carried out using the protocol as reported by Maria (2009). Citrate utilization is used to detect organisms that utilize citrate as a carbon and energy source for growth and ammonium soil as the sole nitrogen source.

This test was identified as defining characteristic that could be used to distinguish between coliforms such as *Enterobacter aerogens* which occur naturally in the soil, on plants and in aquatic environment and fecal coliforms such *as Escherichia Coli* whose presence would be indicates of fecal contamination (Koser, 1999).

The medium was made in slants by dispensing 15 ml of the medium into the MacConkey bottles and then autoclaved at $121^0$c for 15 minutes. The slants were inoculated with the test organisms and inoculated at $35^0$c for 4 days. The medium colour change from green to blue indicated a positive result while no change in colour indicated negative result.

**Catalase Test**

This method was carried out using the protocol as reported by (Karen 2010). In order to survive, organisms must rely on defense mechanisms that allow them to repair or escape the oxidative damage of hydrogen peroxide ($H_2O_2$). Some bacteria produce the enzyme catalase which facilitates cellular detoxification. Catalase neutralizes the bactericidal effect of hydrogen peroxide (Wheelis, 2008) and its

concentration in bacteria has been correlated with pathogenicity.

**Coagulase Test**

The test was used to identify *Staphylococcus aureus* which produce the enzyme coagulase that causes plasma to clot by converting fibrinogen to fibrin. This was carried out by emulsifying a colony of the test isolates with normal saline in duplicate, one serving as control as reported by (Chessbrough, 2000). A drop of the sample was mixed with the suspension and then observed for clumping within 10 seconds. Formation of clumps indicates a positive result while the absence of clumps indicates negative results.

**Indole Test**

This test helps in the identification of *Enterobacter*, which breaks down the amino acid tryptophan with the release of indole. This test was carried out by inoculating the test organism is a medium containing tryptophan. The medium was inoculated as at 35-37$^0$c for up to 48 hours. Indole production was detected by the addition of 0.5ml of kovac's reagent which contains 1-p-dimethylamino paraldehyde found in reagent reacted with the indole for 10minutes to produce a red coloured compound which showed it is positive.

**Vogues Proskauer Test**

1ml of 10% aqueous potassium hydroxide was added to 5ml of culture, and observed at intervals up to 24hours, Anlesosine pink colour indicates a positive result which is owing to the production of acetylmethyl carbinol ($CH_3CH.CHOH.CH_3$) from dextrose. A quicker reaction may be obtained by adding a trace to create the incubated medium and then 2.5ml of 40% sodium hydroxide. The pink colour will develop in few minutes. The test is of value in differently some *Enterobactericeae*

**Oxidase Test**

This test was used to determine whether the oxidase cytochrome in the cultured bacteria organisms in presence and functional. It is used in microbiology to determine if a bacterium produced certain cytochrome oxidase. It uses disk impregnated with a reagent such as N-dimethyl-p-phenylene diamine (DMPD), which is also a redox indicator. The reagent is a dark-blue to maroon colour when oxidized and colourless when reduced. The test is used to see species of organism like *Neisseria, Pseudomonas, Vibro cholera , Acrimonies* etc. The test was carried out on a sterile glass, by placing three (3) impregnated disks in three (3) different positions. Using another glass slide which was flamed and allowed to cool, an inoculum's on the edge of the glass containing the inoculum on the disk was placed and streaked gently. It was allowed for 10seconds and colour change was observed

**Motility Test**

Motility test medium is used to determine the motility of microorganisms. Motility in bacteria can be provided by a variety of mechanisms, but the most common involve flagella.

To test for motility, a sterile needle was used to pick a well isolated colony and stab the medium to within 1cm of the bottom of the tube. It was incubated at 35$^0$c for 18hours unit growth is evident. A proactive motility test is indicated by a turbid area extending away from the line of inoculation. A negative test is indicated by growth along the inoculation line.

**Methyl Red Test**

Sterile glucose peptone water (2ml) was inoculated with the bacteria isolates and incubated at 37$^0$c for 48 hours. After incubation, 4drops of methyl red were added to the cubes. The solutions were homogenized and observed immediately for colour changes. The presence of red colour indicated a positive result while negative result showed change in colour.

**Sugar Fermentation Test**

Bacteria differ widely in their ability to metabolized carbohydrate. However, most bacteria will ferment variety of sugars to form on or more acid end products, which in some cases the accompanied by evolution of gas, usually C02. The acid was dictated by the induction of an indicator in the growth medium. The gas produced was collected in an inverted Durham tubes.

Each test isolate was aseptically inoculated into 1% peptone water containing the test sugars (glucose, lactose, sucrose) and the indicator (bromocresol purple) as well as an inverted Durham tubes inside the liquid medium. The tubes were incubated at 37$^0$c for 24 hours. The medium was examined for the presence of medium was examined for the presence acid production and for gas formation in the Durham tube.

**Isolation and Identification of Fungal Isolates :**
**Identification of Filamentous Fungal**

Filamentous fungi (moulds) produced their characteristic hyphal and reproductive structures during the incubation period, and these were the bases for their identification. They were aseptically picked using a sterile inoculation needle and sub-cultured into fresh Sabrouad Dextrose sugar agar plates to obtain pure cultures.

**Procedure**

The respective mycelia of the isolates were transferred into clean glass slide,greased properly in lactophenol cotton blue solution with an inoculating needle and then careful covered with grease free glass cover slides. They were then microscopically observed under the low, middle, high objectives of the microscope.

**Biochemical Analysis of Samples: pH**

10g of the food sample (Abacha) was weighed and suspended in 100ml of deionized water and mixed thoroughly. The pH was measured using suntex ps-701 pH meter by inserting the pH probe in the mixture. The result was recorded.

**Determination of Copper**

Copper was determined by using Bicin chonate method using H183200 multi-parameter bench photometer at a wave length of 575nm.

10ml of the sample was poured into two (2) separate sample cell bottles. One was used as balnk to zero the photometer and a sachet of H19372-0 Bicinchoniate reagent was added to the second sample all bottle and swirled to mix properly. It was then inserted into the cell compartment and time for 48 seconds. At the end of the count down, the read button was pressed to display the results in mg/l of copper.

**Determination of Iron**

Iron was determined by EPA phenantroline method 315 B, using H183200 multi parameter bench photometer at a wavelength of 525nm.

10ml of the sample was poured into two separate sample cell bottles. One was used as blank to zero the photometer and a sachet of H193721-0 reagent was added to the second sample all bottle and swirled to mix properly. It was then inserted into the all compartment and timed for three (3) minutes. At the end of the count down, the read button was pressed to display the result in mg/l of iron.

**Determination of Potassium**

Potassium was determined using the turbidimetric tetraphenylborate method, using the H183 200 multiparameter bench photometer at a wave length of 610nm.

10ml of the sample was poured into two separate sample bottles. Six drops of H193750100 was added to the sample. One was used as blank to zero the photometer and a sachet of H193750Bi reagent was added to the second sample cell bottle. It was then inserted into the cell compartment and timed for 4 minutes. At the end of the count down the read button was pressed to display the result in mg/l of potassium.

**Results**

The data in table 4.1 shows the total microbial count of the African salad sample from five (5) different locations. The total colonial count (cfu/g) was recorded having the highest count of 9.1 x $10^5$ cfu/g in the sample from Ihiagwa and the lowest count 1.1 x $10^5$ cfu/g in the African salad sample from Umuchima. The total coliform count shows that sample from Obinze has the highest count of 1.5 x $10^6$ cfu/g and the sample from Nekede with the lowest count of 1.6 x $10^A$4. Sample from Ihiagwa had the highest fungal count of 1.7 x $10^3$ cfu/g and sample from Umuchima had the lowest fungal count of 6.5 x $10^2$ cfu/g.

**Table 1: Total microbial counts (cfu/ml) of African salad samples**

| SAMPLE SITE | TOTAL AEROBIC COUNT | TOTAL COLI FORM COUNT | FECAL COLI FORM COUNT | FUNGAL COUNT |
|---|---|---|---|---|
| Nekede | 2.6 X $10^4$ | 1.6 X $10^4$ | 3.2 X $10^4$ | 8.8 X $10^2$ |
| Eziobodo | 2.5 X $10^5$ | 7.2 X $10^4$ | 4.2 X $10^5$ | 1.2 X $10^3$ |
| Obinze | 1.4 X $10^5$ | 6.1 X $10^5$ | 4.5 X $10^5$ | 7.5 X $10^2$ |
| Umuchima | 1.1 X $10^5$ | 1.5 X $10^6$ | 3.4 X $10^5$ | 6.5 X $10^2$ |
| Ihiagwa | 9.1 X $10^5$ | 1.1 X $10^5$ | 4.1 X $10^5$ | 1.7 X $10^3$ |

**Table 2: Occurrence and frequency distribution of micro-organisms isolated from African salad samples**

| ORGANISM | SAMPLES | | | | | FREQUENCY | %OCCURRENCE |
|---|---|---|---|---|---|---|---|
| *Bacillus spp* | + | + | + | + | - | 4 | 16 |
| *Staphylococcus spp* | + | + | - | + | + | 4 | 16 |
| *E.coli* | + | + | + | + | - | 4 | 16 |
| *Streptococcus sp* | + | + | - | - | - | 2 | 8 |
| *Klebsiella spp* | - | - | + | + | + | 3 | 12 |
| *Proteus spp* | - | - | + | - | + | 2 | 8 |
| *Pseudomonas spp* | - | - | - | - | + | 1 | 4 |
| *Mucor spp* | + | - | - | - | + | 2 | 8 |
| *Aspergillus spp* | + | - | + | - | + | 3 | 12 |

Table 3. Summarized the biochemical analysis of the five African salad samples

| S/n | PARAMETERS | OBINZE | NEKEDE | IHIAGWA | UMUCHIMA | EZIOBODO |
|---|---|---|---|---|---|---|
| 1 | pH | 4.6 | 5.8 | 5.7 | 4.5 | 5.2 |
| 2 | IRON (mg/l) | 11.40 | 10.48 | 14.28 | 9.35 | 18.80 |
| 3 | COPPER (mg/l) | 1.71 | 2.42 | 1.39 | 2.53 | 1.52 |
| 4 | POTASSIUM (mg/l) | 525 | 425 | 350 | 380 | 740 |

**Test of Hypotheses**

Table 4. Summary of one way Anova of Total Microbial Count (cfu/ml) of African salad samples

| Source of variation | SS | DF | MS | Fcal | Ftab |
|---|---|---|---|---|---|
| Sample | 2.02x10^A12 | 3 | 6.74539x10A11 | 1.678776 | 3.24 |
| Error | 6.43x10^A12 | 16 | 4.01804x10A11 | | |
| Total | 8.45x10^A12 | 19 | 4.44868x10A11 | | |

Mean values in the column followed by different superscript are not significantly different (P < 0. 05).Therefore there is no significant relationship between the bacterial load from the different sampling areas.

**Discussion**

The International Commission for Microbiological Specification for Foods (ICMSF, 1996) state that ready-to-eat foods with plate counts between 0 - $10^3$ is acceptable, between $10^4$ - < $10^5$ is tolerable and $10^6$ and above is unacceptable.

The high level of contamination of fecal coliform count from African salad sample from Umuchima could be associated to the fact that the African salad requires little or no heat during preparation. Similarly the extensive handling and mixing during processing could have introduced contaminants via food handlers, utensils and from the environment.

Food handling personnel play important role in ensuring food safety throughout the food production, processing, storage and preparation. Mishandling and disregards to hygienic measures on the part of the food vendors have been reported to introduce contaminants and pathogens that survive and multiply in sufficient numbers to cause illness in the consumers (WHO, 2000).

The isolation of *Bacillus subtilis, Saphylococcous aureus, Escherichia coli, Klebsiella spp, Proteus spp, Aspergillus niger* corroborate the findings of Mensah *et al* (2002), in which these organisms were implicated in ready-to-eat foods.

The occurrences of *Bacillus spp* and the moulds could be as a result of prevalence of their spores in environment (Mckillip, 2000). Richard *et al* (2007) showed that gastrointestinal disease has been reported by eating food containing bacillus Spores. *Bacillus subtilis* c auses food poisoning by means of enterotoxins, *Bacillus subtilis* were isolated from the African salad samples..

Feng *et al* (2007) suggested that *Escherichia coli* is part of the normal flora in the colon of human and other animals, but can be pathogenic both within and outside the gastrointestinal tract. Enterotoxigenic *Escherichia coli* (ETEC), this organism is a common cause of “traveler’s diarrhea” in developing countries. It infects only humans with transmission occurring through food and water contaminated with human waste or by person to person. It may also be as a result of poor sanitary habit of the handlers of the African salad, visiting toilets without washing hands, changing baby diapers and not washing hands, sneezing and coughing without covering the mouth (Hobbs and Gilbert, 1978; Mensah *et al*, 1999).

**Conclusion and Recommendation**

The total microbial load of 10 cfu/g sample recorded for TAPC, coliform count and fungal count showed unsatisfactory level according to the ICMSF (1996) specification. The high counts could be associated to the fact that African salad is a product consumed raw with no heat treatment to reduce microbial load.

African salad contains raw vegetables these have been shown to contain high microbial loads of diverse species (Eni , 2010) the personnel involved in the preparation of African salad, the utensils / equipments could have contributed to the microbial load of the product. Sample analyzed in this work were purchased from food vendors .African salad is normally prepared early in the day hawked throughout the day. The product is often left unpreserved and at the ambient temperature day this could encourage microbial proliferation (temperature abuse)

The general public has the right to consume safe and suitable foods. To achieve improved microbial quantity and safety of vendor dispensed African salad and other ready to eat foods sold in sub-urban areas in Owerri, Imo state the following are recommended:

1. Health education for food handlers about food safety
2. Holding educational programs for public to protect them during the handling, preparation, serving and storage of food.
3. Establishing committee to monitor food safety and increase the numbers of trained food inspector
4. Increasing the numbers of analytical services
5. Supporting and encouraging scientific research about food safety.

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