



Culture-Dependent Evaluation of Microbial and Proximate Composition of Ready-to-Eat (RTE) African Salad Sold at Nkpolu-Oroworukwo Ultra-Modern Market (Mile 3, Diobu), Port Harcourt, Rivers State, Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

This study attempts to evaluate culture-dependent microbial and proximate composition of African salad (Abacha) sold at Nkpolu Oroworukwo (mile 3) market, Port Harcourt, Rivers State, Nigeria. Samples were bought from stationary vendors at market within the months of March, 2019 to March, 2021. Garnished African salad (GAS) and ungarished African salad (UAS) samples were determined by standard microbiological techniques whereas proximate composition (PC) was analysed by the methods of Association of Official of Analytical Chemists (AOAC) Total aerobic plate counts (TAPCs), total coliform counts (TCCs) and fungal counts (FCs) of GAS were higher 4.5×10^4 CFU/g, 5.3×10^4 CFU/g and 1.1×10^4 CFU/g respectively as against 3.4×10^4 CFU/g, 3.8×10^4 CFU/g and 0.8×10^4 CFU/g for UAG. Microfloral diversity were more in GAS with species such as *Escherichia coli*, *Bacillus subtilis*, *Klebsiella* sp., *Micrococcus* sp., *Bacillus* sp., *Enterobacter* sp., and *Aspergillus* sp., *Penicillium* sp., *Rhizopus* sp., *Fusarium* sp., *Mucor* sp., *Saccharomyces* and

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Candida species than in UAS. Nutrient composition, energy (116.00Kcal/100g) and pH (6.4) values were higher in GAS. In contrast, only the moisture (62.52±0.53%) and carbohydrate (23.21±0.21%) contents were slightly higher in UAS than those in GAS. The study revealed that African salad' bioburden was slightly above the microbiological standard limits of International Commission on Microbiological Specification for Foods (ICMSF) and (Public Health Laboratory Service (PHLS) for RTE foods for mesophilic aerobic bacteria (MAB)/TAPC but exceeded that for total coliforms. However, the microbes detected are likely aetiologic agents of several food borne diseases (FBDs) in human and animal pathologies. Therefore, adequate surveillance, monitoring and compliance with hygienic practices and environmental sanitary measures during processing, fortification and distribution should be enforced by regulatory agencies and stakeholders for wholesome and palatable RTE foods.

Keywords: Culture-dependent; ready-to-eat; African salad; abacha bioburden; proximate composition.

1. INTRODUCTION

African salad (Cassava-tapioca porridge (Abacha) is derived from Cassava (*Manihot esculenta* Crantz), a basic staple food for an estimated 500 million farmers, countless processors and traders with Nigeria being the largest producer (of Cassava) in the world [1,2,3]. Abacha/Tapioca porridge as a RTE salad, is generally regarded as mixed greens, processed from single or mixed raw ingredients such as vegetables, fish, fruits, spices, potash, palm oil, cooked meat, garden eggs, grains, nuts, etc., with sauce or salad dressing [4,5,6]. The main reasons for street food sales and proliferation of informal markets are associated with the growing demand for cheap, RTE foods and the poverty level of most citizens in most low-and middle-income countries (LMICs). So, these vendors operate without minimum hygiene, regulatory standards and sanitation conditions.

African salad is consumed by a variety of people; school-age children and the fast-paced working class with little or no time to prepare meals as well as a side dish to various Nigerian rice recipes and as a special delicacy during traditional festivals [7,8]. Its economic and social advantage hinges on ease of production, availability, affordability, serviceability and palatability but prone to microbial contamination [9,10]. Processing of African salad necessitates boiling/cooking and fermentation by steeping in potable water for 24h or more to reduce starch and poisonous compounds (e.g., hydrogen cyanide) [8,11] to improve palatability and organoleptic properties prior to consumption. Several researchers have reported poor personal hygiene, lack of education, ingredients, water and sanitary status of the environment as fundamental predisposing factors of microbial contamination of vended RTE foods [12-18]. A

variety of the microbes (e.g., bacteria, fungi and viruses) reported in RTE foods not only accentuate public health risks but also produce potential toxigenic compounds [4, 19-22]. Cross/post-contamination may also be associated with insect vectors [22,23]. Consequently, these contaminants in salad may result in unwholesomeness which may be linked with outbreaks of FBDs [24,25].

In spite of the long history of vended RTE foods as a source of livelihood to many households, there is paucity of data regarding their contribution to the nutritional value of the diet [26] as well as microbiological safety due to variation in fortification by processors. The quality of food can be assessed by the amount of available nutrients and microbiological quality of the final products. Therefore, deliberate efforts to know the quantity and quality of the nutritional contents of vended RTE foods, their contribution to the overall energy and available nutrient intake is pertinent. Additionally, adoption of more proactive approaches in identifying and reducing food safety risks by bridging the information gap with regard to consistent surveillance, monitoring and evaluation of nutritional and microbiological status of vended RTE foods such as African salad is crucial. Though, they have been reported to be a cheap source of energy and protein intake than pre-packaged processed foods [27,28]. This study, however, focuses on culture-dependent evaluation of microbial and proximate composition of African salad as well as assess the level of compliance to microbiological quality standards.

2. MATERIALS AND METHODS

2.1 Description of Study Area

Mile 3 market, Diobu, the study area is in Nkpolu-Oroworukwo community in Port Harcourt City

Local Government Area, Rivers State, Niger Delta, Nigeria. This community is ranked among the most commercially and industrially lively areas in the city. It accommodates foodstuffs, timber and auto-spare parts markets, auto-mechanic workshop, ceramics and tiles shops, motor park, banks, part of *Azienda Generale Italiana Petroli* (Agip) base landing jetty, Eagle island as well as educational institutions (Rivers State University inclusive) and is a high densely populated residential area to Nigerians and other nationals. Mile 3 market also called Nkpulu-Oroworukwo ultra-modern market, lies at coordinates; 4°46'12.796"N (Latitude) and 6°58'24.4866"E (Longitude) Fig. 1.

2.2 Collection of Samples

Freshly prepared garnished African salad (GAS) and ungarnished African salad (UAS) samples (Plates A and B) were purchased at three stationary locations from different vendor at Mile 3 market, Nkpulu-Oroworukwo from the months of March, 2019 to March, 2021 (sampling was truncated by COVID-19 lockdown). All samples were obtained aseptically in sterile sealable take-away containers, package in polyethylene bags and transported to the Microbiology Laboratory of

Rivers State University, Nkpulu-Oroworukwo, Port Harcourt.

2.3 Microbiological Analysis

Serial dilutions were carried out after blending 25g samples of GAS and UAS in 225mL peptone water (Titan Biotech Ltd. Bhiwadi-301019, Rajasthan, India.) to obtain (10^{-1}) a homogenate respectively. Total viable/Aerobic plate counts (TAPCs) were determined on surface-dried nutrient agar (NA) and plates were incubated in duplicates at 37°C for 24hours. Coliforms including *Escherichia coli* colonies were determined on MacConkey' agar (McA) using the spread plate method and duplicate plates were incubated at 37°C for 24hours. Coliforms were further subjected to presumptive and confirmatory tests. Fungal counts (FCs) were determined using an aliquot (0.1ml) portion of serially diluted samples on solidified Sabouraud' dextrose agar (SDA) and spread-plated with a sterilized bent glass rod, followed by incubated at room temperature ($25\pm 2^{\circ}\text{C}$) for 3-5days. Discrete colonies from well-isolated plates showing (30-300) colonies were picked at random, sub-cultured for purification, stored on slopes at refrigeration temperature and used for morphological and biochemical tests [29,30].

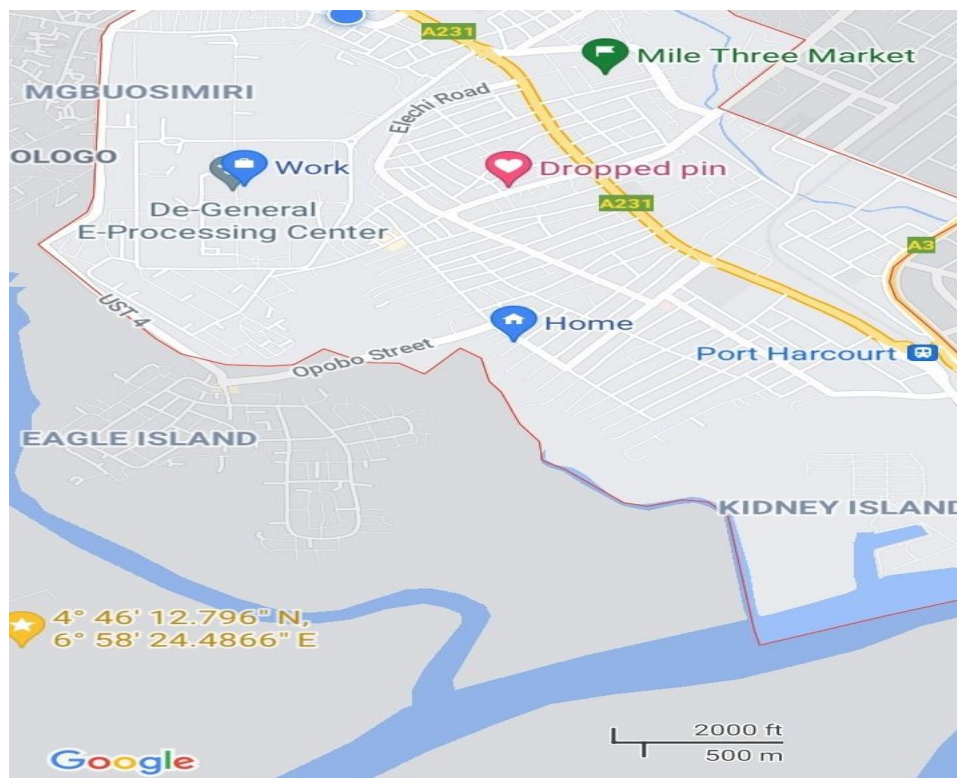


Fig. 1. Sampled site; Mile 3 market, Nkpulu-Oroworukwo, Port Harcourt



Plates A and B. Garnished and Ungarnished African salad respectively

2.4 Identification of Isolates

Identification of bacterial isolates was carried out based on their cultural (appearance; pigmentation, consistency, margin and elevation), morphological (Gram’s reaction, size and shape) and biochemical characteristics (catalase, oxidase, indole, citrate utilization, Methyl red and Voges- Proskauer tests, etc.) as well as sugar fermentation tests [31,32,29].

2.5 Determination of pH and Proximate Composition of African Salad

For pH determination, representative 25 g samples were blended with 50 ml deionised water and the pH measured by dipping a pocket-size digital pH meter (HI 2007, Hanna instrument, USA) after it had been calibrated in buffer solution of pH 7. Proximate composition of GAS and UAS were determined for the following nutrient parameters; moisture, ash, crude protein, crude fibre and fat contents in accordance with AOAC [33,34]. Carbohydrate content was determined by subtracting other parameters from 100; thus, $100 - (\% + \% \text{ Ash} + \% \text{ Fat} + \% \text{ crude protein} + \% \text{ fibre})$. PCs were determined in triplicate and calculated in percentages.

2.6 Statistical Analysis

The experiment was replicate and mean and standard deviation were calculated using Microsoft Excel® 2016.

3. RESULTS

Total aerobic plate counts (TAPCs), total coliform counts (TCCs) and fungal counts (FCs) are presented in Table 1. Higher microbial counts were observed in GAS samples than in those of UAS. This may be attributed to nutrient fortification and environmental conditions.

The percentage frequency of occurrence of bacterial species from GAS are presented in Fig. 2.

The % frequency of occurrence of bacterial species ranged from 40-70 with *E. coli* as the highest, followed by *B. subtilis* and *Enterobacter* sp., the least. Six bacterial species belonging to five (5) genera; three Gram negative (*E. coli*, *Klebsiella* and *Enterobacter* species) and three Gram positive (*B. subtilis*, *Bacillus* and *Micrococcus* species) bacteria were identified (Fig. 2).

Table 1. Total aerobic plate counts (TAPCs), total coliform counts (TCCs) and fungal counts (FCs) (CFU/g) of GAS and UAS samples

(x10 ⁴ CFUg ⁻¹)	GAS	UAS
Bacteria		
TAPC	4.5	3.4
TCC	5.3	3.8
Fungi		
FC	1.10	0.8

Legend : GAS = Garnished African salad; UAS = Ungarnished African salad; CFU = Colony forming unit

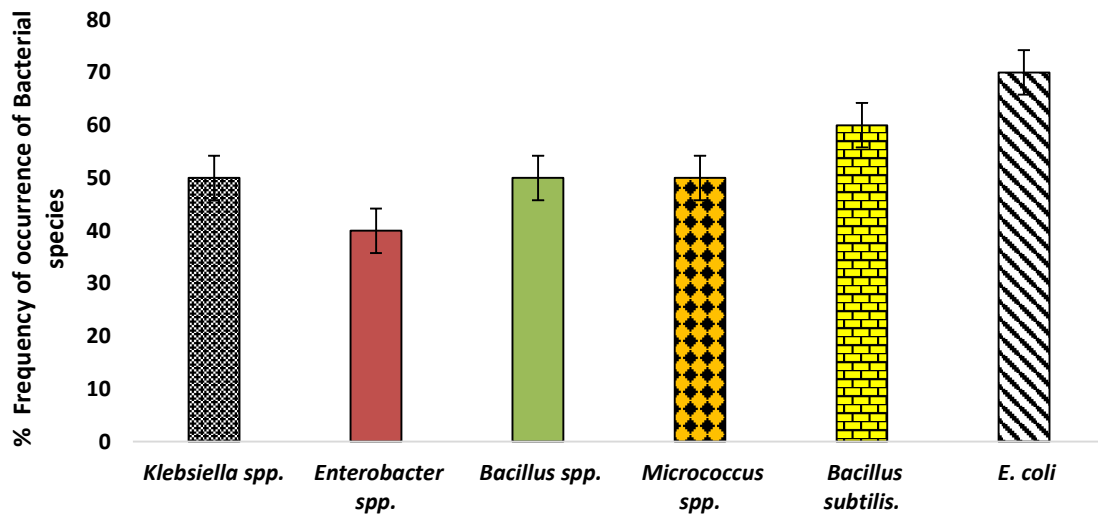


Fig. 2. % Frequency of occurrence of bacterial species from garnished African salad

The percentage frequency of occurrence of bacteria identified from UAS are displayed in Fig. 3. The bacterial species were similar to those of GAS, except that *Enterobacter* sp was absent. The % frequency of occurrence of the species were lower (30-60) but with similar trend to those of GAS above.

The fungal species identified from GAS samples are presented in Fig. 4, with *Penicillium* species having the highest (80%) frequency of occurrences, followed by *Aspergillus* sp (50%) and the lowest being *Candida* species (20%). Seven genera of fungi were identified; 5 moulds

and 2 yeast (*Saccharomyces* and *Candida*) species, Fig. 4.

The percentage frequency of occurrence of fungi identified from UAS are presented in Fig. 5. It indicates that the % frequency was lower, between 20 and 70 depicting similar trend of fungal occurrence in GAS (Fig. 4) except for the absence of *Mucor* sp.

The pH and proximate composition (PC (%)) values are depicted in Table 2. GAS had higher values of pH, crude protein, fat, fibre, ash, and energy whereas UAS were higher in moisture and crude carbohydrate contents.

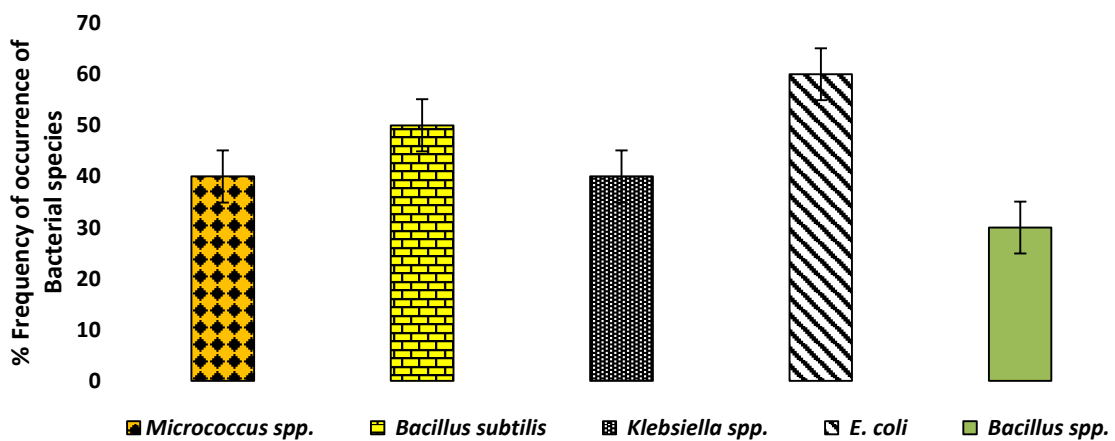


Fig. 3. % Frequency of occurrence of bacterial species from ungarnished African salad

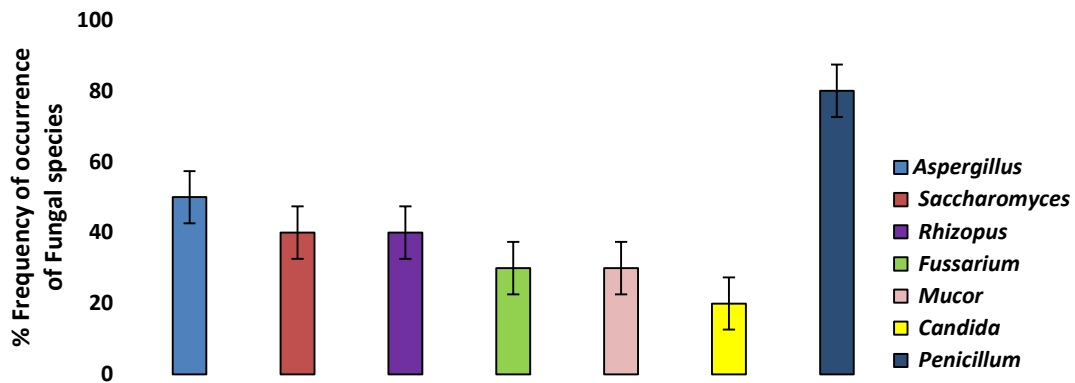


Fig. 4. % Frequency of occurrence of fungal species from garnished African salad

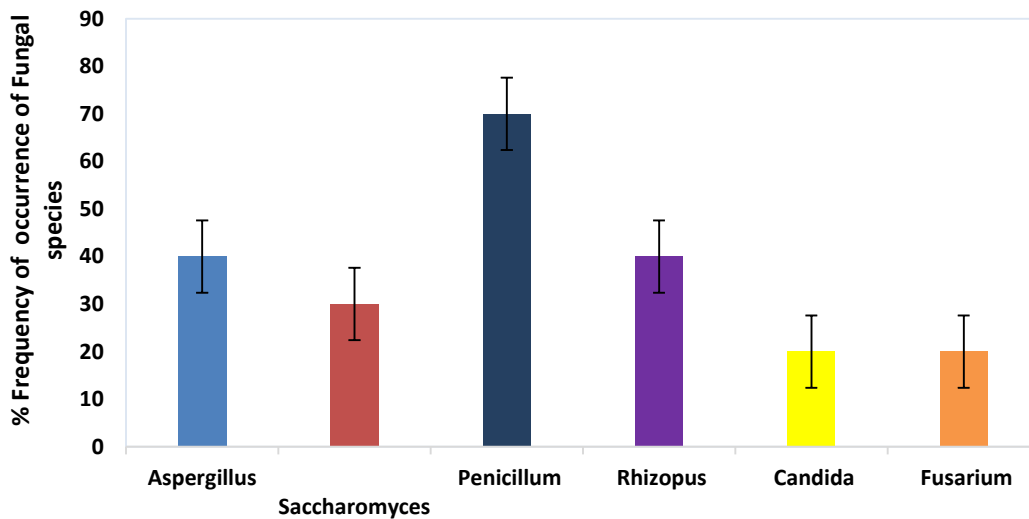


Fig. 5. % Frequency of occurrence of fungal species from ungarnished African salad

Table 2. pH and Proximate composition (%) values of GAS and UAS samples

Parameter	GAS	UAS
pH	6.4	5.2
Moisture content	62.34±0.33	62.52±0.53
Crude protein	4.68±0.53	2.41±0.57
Carbohydrate	21.0±0.06	23.21±0.21
Fat	3.25±0.18	1.22±0.21
Fibre	3.62±0.02	3.42±0.22
Ash	6.81±0.39	4.54±0.42
Energy content (Kcal/100 g)	116.01	114.00

Legend : GAS = Garnished African salad; UAS = Ungarnished African salad

4. DISCUSSION

African salad as a single recipe is a bland/junk food processed from cassava. The microbial load/counts increases based on ingredients used

for fortification, education, hygiene, environmental sanitation as well as time-temperature abuse. Bioburden of the samples were slightly above ($\geq 10^4$ CFU/g) the tolerable standard limits by ICMSF [35] and (PHLS [36] for

MAB/TAPC which corroborates earlier literature reports [12,19], but exceeded limits for coliforms (*E. coli* inclusive) for street vended foods. However, investigators reported much higher counts when Ugba (*Pentaclethra macrophylla*) and Ogiri (*Ricinus communis*) were incorporated into the dish [8,37]. From previous reports, it is apparent that the type of ingredients used to fortify African salad, hygienic practice as well as time-temperature abuse are major indicators of microbial dynamics of this food.

Microbial contamination is frequent in traditional cold foods, due to the way in which foods are prepared, handled, preserved and packaged. The high percentage frequency of occurrence of *E. coli* and coliforms in African salad is phenomenal and consistent with those found in traditional RTE foods in Nigeria and other developing countries [12,24,25,38,39]. Such levels of contamination is suggestive of poor handling, sanitation and possible faecal pollution. The detection of Gram positive bacteria such as *Bacillus* species in African salad from the present study is not uncommon as these organisms have been recovered from RTE foods [19,40,41]. The occurrence of spore formers and moulds could be associated with their ability to survive the processing and adverse environmental conditions. *Escherichia coli*, *Bacillus subtilis*, *Klebsiella* sp., *Micrococcus* sp., *Bacillus* sp., *Enterobacter* sp., and *Aspergillus* sp., *Penicillium* sp., *Candida* spp., *Rhizopus* sp., *Saccharomyces* sp., *Fusarium* sp., *Mucor* sp., reported in the present work concurs with the findings in literature [8,19,42]. Out of this diverse fungal genera the frequently occurring ones in RTEs are *Aspergillus*, *Fusarium*, *Mucor*, *Penicillium*, and *Rhizopus* [4].

The microbial diversity and other differences between GAS and UAS could be attributed to added ingredients, hygiene, pH and nutritional composition. The nutrient composition of GAS revealed that it was richer than UAS in crude protein, fat, ash, fibre contents as well as energy. The relatively high moisture contents of both samples may have encouraged microfloral proliferation and diversity as well as decreased shelf life. The high energy value of African salad may be due to added ingredients which by extension account for the nutritional quality as well. The nutrient components reported are similar to those earlier recorded [8] Thus, this study has demonstrated the nutritional, energy and microbiological risk assessments of African salad as well as the beneficial impacts of

complementing our diet if consumed in adequate proportion.

5. CONCLUSION

African salad is nutritious, consumed fresh and without application of chemical preservatives. The microbial diversity and energy value of African salad depends on the raw material-base and added ingredients. Adequate processing, sanitization of utensils, hygienic practice, education and compliance with environmental sanitary measures would result in an African salad that will meet food standard regulatory policies.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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