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Quality Aspects of African Salad

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Abstract
The proximate and microbiological quality of African salad a special salad recipe native to Nigeria was investigated in order to provide scientific, research based information on the nutritional and micro floral composition of this exotic delicacy. Samples of salad were purchased from six food vending sites which serve as the major business and residential area in Owerri. The predominant bacterial isolates from African salad belong to *Bacillus spp, Staphylococcus spp, Escherichia coli, Enterococci* and *Serracia*. The fungal spp isolated include *Saccharomyces, Mucor, Rhizopus, Penicillium* and *Aspergillus*. The mean total aerobic plate count range from $7.7x10^{11}$ to $4.8x10^{12}$, coliform count range from $8.5x10^{10}$ to $3.5x10^{11}$ and fungal count range from $6.6x10^{9}$ to $4.7x10^{10}$. Based on the specifications by International Commission for Microbiological Specification for Foods (ICMSF), the level of contaminations was unacceptable and could pose health challenge. The chemical composition of the African salad samples consist of carbohydrate (21.07%), protein (4.38%), fat (7.80%), fiber (3.29%), and moisture (62.72%). Energy value of 169.30 kcal and minerals were recorded. African salad as seen from this report is a nutritious food, fit for all age groups. Special care however, has to be taken in its preparation as contaminants could be introduced through the ingredients. Education of food handlers and the general public on food safety measures, effective Hazard Analysis Critical Control Point (HACCP) application and Good Manufacturing Practices (GMP) implementation is imperative.

Keywords: GMP; HACCP; microbiological quality; proximate quality; salad.

INTRODUCTION

African salad popularly called "Abacha, Abacha Ncha, Abacha and Ugba" by Igbo tribe of Nigeria, it 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 and 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 (African salad: Abacha & Ugba, 2013) 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 (Abacha Ncha: African salad, 2012).

Abacha is processed by harvesting cassava tubers, after which they are peeled, washed and cooked. These are then shredded into fine thin slices, and soaked overnight for fermentation so as to thoroughly reduce the starch and hydrogen cyanide from the cassava. The shredded and fermented cassava is again thoroughly washed the following day before drying it for 2-3 days (African salad: Abacha & Ugba, 2013).

The preparation of African salad takes great efforts and the ingredients needed to prepare African salad 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 (African salad: Abacha & Ugba, 2013). 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 (African cassava salad-Tapioca, 2006; Miriam and Anthonio, 2011; Nigerian Appetizer-Abacha: African salad, 2012; Maky, 2013; 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) (African cassava salad-Tapioca, 2006; Miriam and Anthonio, 2011; Nigerian Appetizer-Abacha: African salad, 2012; African salad, 2013; Osewa, 2013).

There is paucity of information on the chemical and microbiological compositions of African salad as consumed. Though several works abound on some of the component ingredients, the notion that African

salad is nutritionally rich is only but speculative based on its component ingredients. The objective of this work is to carry out microbiological and proximate analysis of African salad as prepared by food vendors in Owerri, Imo state, Nigeria. With a view to stirring and stimulating further research and thus building a compendium on the diversities in compositions of different preparations of this all important African dish.

MATERIALS AND METHODS

Sample Collection

Fifteen samples of African salad were purchased from different vendors in Eke-Ukwu Market, Central (Okigwe) motor park, Douglass road, Amakofia, Nekede and Ihiagqwa all in Owerri, Imo State, South-East Nigeria. The samples were collected in sterile specimen containers and were transported in cold pack to the laboratory for analyses within 30 to 45 minutes of collection.

Microbiological Analysis of Samples

Ten gram (10g) samples were serially diluted 10^{-1} to 10^{-6} in sterile peptone water and aliquot 0.1ml volumes of each sample homogenate inoculated by spread plate method onto freshly prepared Nutrient agar, MacConkey agar and Potatoes Dextrose agar[PDA] (all from Biolab, Hungary), for Total Aerobic Plate Count, Coloform count and Fungal count respectively. Approximate 1g samples were inoculated into lactose broth in capped test tubes with inverted Durham tubes for coliform test. Manithol Salt agar, MRS agar, M17 agar, Bismuth sulphite agar (Biolab, Hungary) were inoculated with 0.1ml of samples homogenate for isolation of *S. aureus, Lactobacillus spp and Streptococci*. Bismuth sulphite agar was inoculated for salmonellae after pre-enrichment in selenite broth (Biolab, Hungary). All culture plates were incubated at 37°C for 24 to 48h except however, PDA for fungal isolation which was incubated at 28±2°C for 3 to 5 days. At the expiration of incubation period, culture plates were examined for enumeration and cultural identification of colonies.

Enumeration and Characterization of Microbial Isolates

Colony counts were with digital colony counter (Gallenkamp, England) and total population expressed as colony forming units per gram (Cfu/gm). Pure culture of isolates obtained by repeated subculture on freshly prepared nutrient agar was stored on slants at 4°C refrigeration temperature for further identification. Identification of characteristic bacteria isolates was based on colonial morphology, microscopy and biochemical tests (Speck, 1976; Jolt *et al.*, 1994). Fungal isolates were identified based on morphological characteristics, pigmentation on media and microscopy with reference to standard atlas and manuals (De Hoog *et al.*, 2001; Tsuneo, 2010).

Coliform Test

Approximate one gram of each sample was inoculated into sterile McCartney bottles containing lactose broth and inverted Durham tubes. Incubation was for 24-48h at 37ºC. Tubes showing gas production and/or colour change of dye were noted as positive for Presumptive coliform test. Confirmatory coliform test was performed by streaking out positive presumptive tests on duplicate Eosin Methylene Blue (EMB) agar plates; incubation was at 37ºC and 44ºC for 24h respectively. Growth of characteristic colonies on EMB constitute positive confirmatory test. Colonies from EMB plates were Gram stained and inoculated into tubes containing lactose broth with inverted Durham tubes for **completed coliform test** and onto Nutrient agar slants for further characterization. Inoculated tubes and slants were incubated for 24h at 37ºC. Gas production and/or colour change of dye plus Gram negative non-spore bearing rod was recorded for presence of coliform. If and when growth at 44ºC is positive, feacal coliform is confirmed (Speck, 1976; Oranusi *et al*., 2004).

Chemical Analysis of Samples

The proximate compositions of the samples were determined according to the procedure outlined by the Association of Official Analytical Chemists (AOAC, 1980). The food samples were analyzed for moisture content, carbohydrate, protein, lipid, ash, fiber, energy content and minerals. Total nitrogen was determined by MicroKjedalh method and the protein value was derived from the nitrogen content by multiplying by a factor of 6.25. Lipid was by the use of Soxhalet extraction, while carbohydrate content was assayed by difference (Nwanze *et a*l., 2006). Energy values were determined by calculation from the individual nutrient content and employing the conversion factors of 4, 9, and 4 for carbohydrate, lipid and protein respectively, as stipulated by FAO/WHO/UNU (1985) and McCance and Widdowson (1993). Minerals were assayed by the use of Atomic Absorption Spectrophotometer (AAS).

RESULTS

The total colony count for bacteria and fungi obtained in the different samples from sampling locations is shown in Table1. All the samples had Total Aerobic Plate Count (TAPC), fungal count and Coliform counts above the tolerable 10^6 limit in foods. The locations for sample collection tends not to have influence on the level of microbial contamination of products as there seems to be no significant difference in levels of contamination of the samples. Coliform test reveals the presence of feacal coliforms able to tolerate and grow at 44°C. Table 2 presents the microbial isolates from the different samples. It reveals the major bacterial isolates to belong to

Bacillus spp, Staphylococci, Escherichia coli, Enterococci and *Serracia*. The fungal spp isolated include *Saccharomyces spp, Mucor spp, Rhizopus spp, Penicillium spp* and *Aspergillus spp.*

The chemical composition of the African salad samples are shown in Table 3. It is rich in basic food nutrients of carbohydrate (21.07%), protein (4.38%), fat (7.80%) and fiber (3.29%). Energy value of 169.30kcal and minerals were recorded.

DISCUSSION

The total microbial load of greater than 10^6 Cfu/g sample recorded for TAPC, Coliform count and fungal count is above the ICMSF (1996) specification for tolerable level of microbial load in foods. 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. The addition of fermented products like 'Ugba' and 'Ogiri' could have contributed immensely to the high microbial load. 'Ugba' and 'Ogiri' has been reported to have high microbial load (Obeta, 1983; Isu and Njoku, 1997; Nwagu *et al.*, 2010). Microorganisms involved in 'Ugba' and 'Ogiri' fermentation was reported to include predominantly *Bacillus, Micrococcus, and Lactobacillus.* Other organisms also isolated from 'Ugba' and 'Ogiri' include *Pseudomonas, Staphylococcus, Enterobacters, Leuconostoc, Corynebacterium, Proteus, E. coli and Alkaligenes* (Isu and Njoku, 1997; Enujiugba, 2009). 'Ugba' and 'Ogiri' as component ingredients of African salad could be the source of these organisms in the samples analyzed.

African salad contain raw vegetables, these have been shown to contain high microbial loads of diverse spp (Eni *et al*., 2010; Oranusi *et al.*, 2013). The personnel involved in the preparation of African salad, the utensils/equipments, water for processing and the processing environment could have contributed to the microbial loads of the product. Samples analyzed in this work were purchased from food vendors; salad is normally prepared early in the day and hawked throughout the day. The product is often left unpreserved and at the ambient temperature of the day, this could encourage proliferation and thus high load of microorganisms above 10^6 Cfu/g foods as recorded in this report. The holding of food over a long period of time at temperature range that could encourage microbial proliferation (Time-Temperature abuse) have been reported as a measure that encourage food borne infections and intoxication by *Staphylococcus aureus* and *Bacillus cereus* (Mensah *et al.*, 1999; Oranusi *et al*., 2007b). During display for sales, the product is opened as often as possible to attend to customers; this practice could contribute to microbial contamination of the salad.

Staphylococcus aureus, Bacillus cereus, Enterococci, E. coli, and fungi such as *Mucor spp, Rhizopus spp, Penicillium* and *Aspergillus spp* have been isolated from foods, food handlers, food utensils and contact surfaces, the presence of these organisms in African salad and microbial counts above acceptable standard limits calls for concern as these organisms have been implicated in diverse food borne infections and intoxications (Oranusi *et al*., 2012). Although the presence of *Salmonelae*, a major human pathogen was not identified, the presence of *Enterococcus faecalis* and *Escherichia coli* is regarded as objectionable as it implies a possible faecal contamination and the possibility of food borne illness due to enteric pathogens. It may also be as a result of poor sanitary habit of the handlers of the African salad, visiting toilet without washing hands, changing baby dippers and not washing hands, sneezing and coughing without covering the mouth (Hobbs and Gilbert, 1978; Mensah *et al.*, 1999). *Bacillus* spp are common contaminating microorganisms isolated from a wide variety of foods and environments. The occurrences of *Bacillus* spp and moulds could be as a result of prevalence of their spores in environment (Mckillip, 2000; Osuntogun and Aboaba, 2004; Amusa *et al*., 2005).

Nutritional analyses of the African salad present the moisture content to be high (62.72%). High moisture content encourages microbial growth on food materials (Onimawo and Egbekun, 1998), the high moisture content of African salad also will make its preservation difficult. The high carbohydrate content could be attributed to the addition of tapioca. This high carbohydrate value makes African salad a good energy food. The protein value could be attributed to the Crayfish and fermented 'Ugba' and 'Ogiri' often added. More so fish and meat are sold separately by the vendors and thus were not included in this analysis. Palm oil is a major ingredient in African salad; this could be responsible for the high lipid content. High lipid content with high moisture increases the chances for rancidity in any food and thus decreases the shelf life of food samples (Asiedu, 1998). The low ash content is indicative of low mineral content. Ash content of a food product influences its quality (Ihekoronye and Ngoddy, 1985). The fiber content of 3.39% depicts that African salad is beneficial because fiber in food helps elimination of bile acids, lower body cholesterol pool and creates variation in faecal bulk and transit time (Ihekoronye and Ngoddy, 1985). The high energy value recorded for African salad could be attributed to the carbohydrate, protein and fat contents, African salad can meet the energy and nutritional need of an individual if eaten in sufficient quantity (Oranusi *et al*., 2007a).

CONCLUSION

The diverse ingredients as may be added to African salad in its preparation will determine the chemical composition of the product and to a large extent the

microbial flora because the different ingredients contribute immensely to the general microbial load as may be presented.

This work has for the first time presented a scientific fact on the microbiological and chemical quality of African salad, it has not presented the diverse variations in the quality of African salad based on different ingredients composition. This will serve as bases for research into this product and thus building of a compendium on the composition of this all important African dish. African salad as seen from this report is a nutritious food, consumed fresh, without preservatives and thus it is recommended for all age groups. Special care however, has to be taken in its preparation as contamination could be introduced through the ingredients used and proliferation of contaminants is easy due to its rich nutrients and conducive environment. Education of food handlers and the general public on food safety measures, effective Hazard Analysis Critical Control Point (HACCP) application and Good Manufacturing Practices (GMP) implementation is imperative.

Table1: Mean total microbial counts Cfu/g of African salad

Sample site	TAPC	Coliform Count	Fungal Count
EKUM	4.8×10^{12}	3.5×10^{11}	3.7×10^{10}
CMPK	8.7×10^{11}	8.5×10^{10}	9.6×10^{9}
DGRD	3.8×10^{12}	2.5×10^{11}	3.8×10^{10}
IHGW	7.7×10^{11}	9.5×10^{10}	6.6×10^{9}
NKED	8.7×10^{11}	8.5×10^{10}	9.6×10^{9}
AMKF	3.8×10^{12}	2.7×10^{11}	4.7×10^{10}

EKUM: Eke ukwu market; CMPK: Central motor park; DGRD: Douglass road; IHGW: Ihiagwa; NKED: Nekede; AMKF: Amakaofia

Table 2. Microorganisms isolated from samples from different sampling sites

Sample site	Microorganisms isolated from samples
EKUM	*Bacillus spp; Staphylococcus spp; Bacillus subtilis; Enterococcus faecalis; Serracia spp; Escherichia coli; Saccharomyces spp; Mucor spp; Rhizopus spp.*
CMPK	*Pseudomonas spp; Bacillus cereus; Enterococcus spp; Escherichia coli; Staphylococci, Rhizopus spp; Penicillium spp; Aspergillus spp; Geotricum spp; Alternaria spp.*
DGRD	*Bacilus spp; Bacillus subtilis; Enterobacter spp; Serracia sp; Streptococci; Fusarium spp; Saccharomyces spp; Aspergillus spp.*
IHGW	*Klebsiella spp; Bacillus spp; Enterococcus spp; Serracia spp; Escherichia coli; Staphylococci;* Saccharomyces spp; Mucor spp; Rhizopus spp.
NKED	*Bacillus cereus; Micrococcus spp; Bacillus subtilis; Proteus spp; Escherichia coli; Candida spp; Saccharomyces spp; Mucor spp; Aspergillus spp.*
AMKF	*Bacilus cereus; Bacilus. subtilis; Enterococcus faecalis; Serracia spp; Escherichia coli; Actinomycetes; Rhizopus spp; Aspergillus spp; Penicillium spp.*

EKUM: Eke ukwu market; CMPK: Central motor park; DGRD: Douglass road; IHGW: Ihiagwa; NKED: Nekede; AMKF: Amakaofia

Table 3: Proximate composition of African Salad

Content	Percentage composition (%)
Moisture	62.72
Carbohydrate	21.07
Crude protein	4.38
Fat	7.50
Ash	1.05
Fiber	3.29
Calcium	34.00(mg)
Iron	7.50 (mg)

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