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Evaluation of Microbial and Nutritional Qualities of Aniga and Epiti Moin: Prestige Foods of South Eastern Nigeria

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Abstract: Investigation on the microbiological and nutritional qualities of aniga and epiti moin moin two cultural foods of South eastern Nigeria reveals that the TAPC of aniga ranged from 1.40 x 104 to 1.70 x108cfu/g, the coliform count was < 10 to 2.70 x 104cfu/g and fungal count was 1.60 x 103 to 1.90 x 108cfu/g. Epiti moin moin had a TAPC of 3.40 x 105 to 4.50 x 1010cfu/g, coliform count of 2.00 x 103 to 1.10 x 105cfu/g, and fungal count of 1.17 x 105 to 1.60 x 108cfu/g. The predominant bacterial and fungal isolates included species of Bacillus, Enterobacter, Aspergillus and Saccharomyces. However, species of Corynebacterium, Mucor, and Penicillium were also recovered from epiti moin moin. Varied concentrations of the phytochemicals saponin, tannin, alkaloid, flavonoid, oxalate and cyanide were detected and the proximate analysis shows that aniga and epiti moin moin had moisture contents of 68.55 and 68.41%, Carbohydrate contents of 23.17 and 21.61%, Protein contents of 5.08 and 5.67% and Fat contents of 1.73 and 2.87% respectively. Aniga and Epiti moin moin are rich in nutrients and phytochemicals; they are foods that could be used to augment the nutritional need of man. It is advanced that the application of good manufacturing practices (GMP) and effective hazard analysis critical control point (HACCP) in the production of these foods will be necessary to curtail microbial contaminants, standardize the processing procedures and thus optimize the foods for wider consumer acceptability.

Key words: Cultural foods, Aniga, Epiti moin moin, Prestige food, HACCP, GMP

INTRODUCTION

Food has been defined as any substance that provides the nutrients necessary to maintain life and growth when ingested[1]. The U.S. Food and Drug Administration defined food as raw, cooked, or processed edible substance, ice, beverage, or ingredient used or intended for use or for sale in whole or in part for human consumption, or chewing gum[2]. Food is classified as one of the factors of life that is not of natural order but of artificial order because, man seek to create his own food specific unto himself or chooses his food, according to criteria linked either to the economic and nutritional dimensions or to the symbolic value with which food itself is invested[3]. Food is culture because it is a decisive element of human identity and as one of the most effective means of expressing and communicating that identity[er]. Food serves various functions in human lives

depending on the culture that man constructs and upholds about a specific food(s). Montanari[3] and Ayeomoni[4] observed that food is a source for pleasure, comfort and security. It is also a symbol of hospitality, social status, and religious significance.

Culturally, food has been classified/categorized as cultural super foods, prestige foods, body image foods, sympathetic magic foods, physiologic group foods[5] or as core foods, complementary foods, secondary foods and peripheral foods[6].

Aniga and Epiti moin are foods that denote different cultural facets of the South- eastern Nigerians. They can be grouped as prestige/secondary foods not because they are expensive or protein based, but because they are widely but rarely/less frequently consumed.

Epiti moin is a steamed maize-plantain pudding made from a mixture of ripe plantain (Musa paradisiaca), hand milled maize (Zea mays), onions, crayfish and fresh ground peppers. Other ingredients can be added depending on choice and availability. It is a protein-carbohydrate rich food that serves different cultural interest in the Southern and Western Nigeria. Aniga is prepared from grated Indian Cocoyam (Colocasia esculenta) mixed with food ingredients and heavily spiced to taste. In some community, Aniga is often prepared in periods of food scarcity between planting and harvest seasons.

Exploring Nigeria huge tourism potential as part of government effort to reduce her over dependence on fossil fuel will automatically mean exposing our rich cultural food heritage to the world. A good understanding, documentation, packaging and presentation of our rich and enormous cultural diversity are necessary. Food is culture and a sound knowledge of the composition of our rich food diversity is imperative, specifically in our highly westernized society where indigenous food culture is eroding fast. This study seeks to evaluate the microbial and chemical composition of Aniga and Epiti moin with a view of creating awareness and interest in these prestige foods.

MATEREALS AND METHODS

Collection and Preparation of Samples for Microbiological Examinations

The samples were obtained from Anambra, Enugu and Imo states of South east Nigeria, between the months of May to July, 2014. Samples of Aniga were obtained from Ekwulobia in Anambra state, Anara in Imo state and Nsukka in Enugu state. Samples of Epiti moin' were purchased from Ihiagwa and Mbieri markets both in Imo state. All samples were labeled and transported in cold chain to the laboratory for analysis. Standard microbiological techniques were adopted for isolation and identification of microbes. Ten grams of each sample for microbiological evaluation was aseptically homogenized in 90 mL of normal saline and tenfold dilution made up to 10^{-6}.

Isolation and enumeration of microorganisms

Aliquot 0.1 mL appropriate dilutions of sample homogenates were plated in duplicate by spread plate technique onto different media prepared in accordance to the manufacturer's instruction. Nutrient agar (NA), (MA) MacConkey agar (Fluka, Germany) and (PDA) Potato Dextrose Agar (Biolab, Hungary), were inoculated for total aerobic plate count (TAPC), coliform count and fungal count respectively. Eosin Methylene blue (EMB) broth (Oxoid, England) in test tubes with inverted Durham's tube was inoculated with one gram test samples for coliform tests, while Salmonella-Shigella agar (Fluka, Germany) was inoculated after 24 h pre-enrichment of samples in Selenite F-broth, for isolation of salmonellae.

Plates of PDA were incubated at 28±3°C for 3 to 5 days while other media plates were incubated at 37°C for 24-48h. Colonies formed were enumerated using digital colony counter (Gallenkamp, England), counts were expressed as $cfug^{-1}$ of sample. Characteristic discrete colonies on the different media were isolated, and purified by repeated sub-culturing on Nutrient agar. Pure cultures were stored on agar slants at 4°C for further characterization.

Coliform test

Tubes of EMB broth with gas formation in Durham tubes and or colour change in broth (positive for presumptive coliform test) were inoculated onto EMB agar (Oxoid, England) for confirmatory and completed coliform tests[7].

Identification of isolates

Bacteria isolates were identified based on morphological characteristics, microscopy, and biochemical tests[8] and using Analitical Profile Index biochemical test kits Biomerieux ® SA. Identification of fungal isolates was based on morphological characteristics of culture and microscopy with reference to standard identification keys and atlas[9, 10].

Chemical analysis of samples

The samples were assayed for protein, carbohydrate, lipid, ash, fiber and moisture following the Association of Official Analytical Chemists (AOAC) standard methods[11]. Protein was obtained from total nitrogen determined by Micro-Kjedalh method and by multiplying with a factor of 6.25. Carbohydrate content was assayed by difference; lipid was by the use of Soxhalet extraction while ash and moisture were determined from oven dried samples and use of muffle furnace. Crude fiber was derived from weight of sample on incineration.

Standard methods were followed in the assay for some phytochemicals; Flavonoid was determined by the method of Zhishen et al., [12] following the descriptions by Hasan et al.[13]. Alkaloid was by the alkaline precipitation method by Harborne[14]. Cyanide was determined by the method as described by Anhwange et al. [15]. Saponin was determined by the method as described by Obadoni and Ochuko[16]. The method of Oke[17] as described by Sarkiyayi and Agar[18] was adopted for oxalate determination; Tannin was assayed following the method as described by[18].

Statistical Analysis

The chemical compositions and microbial counts from replicate samplings and on each media type were presented as mean and analyzed with Chi-square test and employing Duncan Multiple Range Test (DMR) to determine level of significance at P= 0.05

RESULTS

The mean total microbial counts of samples examined are presented in Table 1. It reveals that the samples have high microbial load with TAPC ranging from 3.40 x 10^6 to 4.50 x 10^{10} $cfug^{-1}$. Coliform count range from 2.00 x 10^3 to 1.10 x 10^5 $cfug^{-1}$, while fungal count ranges from 1.17 x 10^5 to 1.90 x 10^8 $cfug^{-1}$. Table 2 shows the microbial isolates from samples, predominant microbial species included Bacillus, Enterobacter, Aspergillus and Saccharomyces. Species of Corynebacterium, Mucor, and Penicillium were also recovered from epiti moin.

The chemical compositions of the food samples are shown in Table 3. The saponin content was 1.60 mg/100g for aniga and 1.40 mg/100g for epiti moin. However, epiti moin exhibited a higher flavonoid content of 3.5 mg/100g. Cyanide content mg/100g was 2.42 for aniga and 2.15 for epiti moin. Proximate compositions of the food samples as presented in Table 3 indicated that aniga and epiti moin have low fat, high moisture and carbohydrate contents.

Table 1. Mean total microbial counts $cfug^{-1}$ sample

Sample code	Total aerobic plate count	Coliform Count	Fungal Count
ANA	1.40 x10^4	< 10	1.60 x 10^3
ANE	1.70 x10^8	2.70 x 10^4	1.90 x 10^8
ANN	3.20 x 10^6	1.43 x 10^2	2.45 x 10^6
EPI	3.40 x 10^5	2.00 x 10^3	1.17 x10^5
EPN	2.10 x 10^{10}	1.10 x 10^5	1.40 x 10 ^{8}
EPI2	4.50 x 10^{10}	6.00 x 10^3	1.64 x 10^8

Key: AN = Aniga samples; EP = Epiti moin samples

Table 2. Microbial isolates from Aniga and Epiti moin

Food Sample	Microbial isolates
Aniga	B. cereus, Enterobacter spp, Enterococcus, Aspergillus niger, Serratia spp, Saccharomyces cerevisiae, Saccharomyces ellipsoideus
Epiti moin	B. subtilis, B. cereus, S. aureus, Enterobacter spp, Aspergillus niger, Corynebacterium, Saccharomyces cerevisiae, Micrococcus spp, Mucor spp, Penicillium spp

Table 3. Chemical compositions of Aniga and Epiti moin

Food sample	Proximate Compositions (%)					
	Moisture	Fat	Fibre	Protein	Ash	Carbohydrate
Aniga	68.55	1.73	0.47	5.08	1.00	23.17
Epiti moin	68.41	2.87	0.07	5.67	1.37	21.61
	Phytochemical composition (Mg / 100g)					
	Saponin	Tanin	Alkaloid	Flavonoid	Oxalate	Cyanide
Aniga	1.60	0.21	2.90	2.80	0.71	2.42
Epiti moin	1.40	0.17	2.80	3.50	0.66	2.15

DISCUSSION

The mean TAPC presented for epiti moin and aniga indicated contamination of the samples beyond tolerable limit. ICMSF[19] described heterotrophic microbial food contaminants as acceptable at levels of ≤ 10^3, tolerable at 10^4 to 10^5 and unacceptable at >10^6 cfug^{-1}. Heterotrophic microbial contaminants are often post process contaminants introduced from food processing environment or contaminants associated with raw materials that survived the cooking temperature as heat resistant spores[20]. The presence of coliforms in the food samples is an indication of post process contamination and poor sanitary practices in the handling of these samples. Coliforms are indicator organisms signifying contamination of a product by fecal matter, via possible use of polluted water, utensils/equipment or the personnel that handled the food from processing, through packaging, transportation to sales[19, and 20]. The high fungal count could be associated to the fact that moulds are spore bearers and common environmental contaminants. The wrapping materials, food handlers, utensils and surrounding air are often common source contaminants for fungal spores in food products.

The predominance of Bacillus, Enterobacter, Aspergillus and Saccharomyces species in aniga and epiti moin corroborate the observations that diverse species of microorganisms have been reported in ready to eat foods[21-23]. Bacillus and Aspergillus species are spore formers and are ubiquitous, most bacillus species are food spoilage organisms[24], some have served useful purposes for man as antibiotics producers[25-27], producers of useful chemical substances and pesticides[28, and 29], used as probiotics[30-32] and in food fermentation[33-35]. Some bacillus species however, are known to be opportunistic pathogen or pathogens[24, 36]. The presence of bacillus spp in aniga and epiti moin moin must therefore be weighed against the potentials of the individual organisms.

Some fungi are useful to man[37-42]; a good number of fungi are agents in fruits/food spoilage[43-46]. The yeasts Saccharomyces are saprophytic fungi often associated with fermentation and spoilage in sugar rich foods, juice and condiments. Their presence in epiti moin moin and aniga could be attributed to

contamination from the raw materials, environment and it is in agreement with reports of its isolation from diverse food sources[47-49]. Some fungi produce deleterious mycotoxins[50-52], yet some others are pathogen or opportunistic pathogen in human mycosis[53-56]. Fungi contaminants in foods must therefore be handled with caution and not just as mere contaminants.

The presence of Enterococcus and Enterobacter in some of the samples indicates poor sanitary standard post processing. They are an indicator of faecal contamination[57]; Enterococci are mainly used to monitor distant time contamination[58]. Klein[59] however, noted that Enterococci are present in many foods and agricultural soil added with manure thus is also considered as normal food microflora. The food handlers and water used for food processing are likely the major source of this organism in aniga and epiti moin moin.

The chemical composition of aniga and epiti moimoi consists of saponin, tannin, alkaloid, flavonoid, oxalate and cyanide in various concentrations. These phytochemicals could, depending on concentration, present as either nutritional or anti-nutritional component of food. The saponin contents of aniga and epiti moin moin observed in this report is in tandem with earlier presentations on some traditional foods by Amadi et al.[60]. Saponins are reported to have antifungal, antiviral, antibiotic and anthelmintic activities[61-64]. Saponins have hypocholesterolaemic action, thus may prove useful in the control of human cardiovascular disease[65].

Flavonoids have also been shown to be capable of modifying low density lipoproteins (LDL) in order to greatly increase its uptake by macrophages, thereby reducing the level of low density lipoproteins (LDL) in the body[66]. As such, flavonoids can be applied in the management of atherosclerosis[67]. Robbins[68] and Srinivasan et al., [69] reported the beneficial physiological and pharmacological effects of flavonoids on blood capillaries. The action of flavonoids on erythrocyte aggregation is consistent with their beneficial effects on capillaries and in disease states because the aggregation impairs microcirculation and also induces pathology[14].

Tannins are constituents of several drugs because of their astringent property. They are used in the treatment of haemorrhoids, diarrhoea, dysentery, leucorrhoea and as a useful medicine for throat infections[70]. Tannins in general are considered to be part of the plant chemical defenses against pathogens and herbivores[71]. There are quite a number of anti-nutritional effects of the tannins present in plants. The mechanism of these effects is understood by their ability to form a less digestible complex with dietary proteins. They may as well bind and inhibit the endogenous protein, such as digestive enzymes[72]. The precipitation of protein-tannin complex depends on pH, ionic strength and molecular size of tannins. Molan et al.[73-75] reported the inhibitory effects of tannins against gastrointestinal nematodes and deer lungworms. They reported that condensed tannins (CT) extracted from forages have the ability to inhibit the development of Trichostrongylus colubriformis eggs (L1) to infective larvae (L3) and to reduce larval motility.

Alkaloids are known to have pharmacological effects and are used as medications, as recreational drugs, or in entheogenic rituals. Examples are the local anesthetic and stimulant cocaine; the psychedelic psilocin; the stimulant caffeine; nicotine; the analgesic morphine; the antibacterial berberine; the anticancer compound vincristine; the antihypertension agent reserpine; the cholinomimeric galantamine; the spasmolysis agent atropine; the vasodilator vincamine; the anti-arhythmia compound quinidine; the anti-asthma therapeutic ephedrine; and the antimalarial drug quinine. Alkaloids have been used in the treatment of skin infections[76].

Most cyanide is highly toxic, and antinutritional, cyanide ion halts cellular respiration by inhibiting cytochrome C oxidase enzyme in the mitochondria. This stops ATP formation, tissue suffers energy deprivation and death follows rapidly[77]. Cyanide has teratogenic effects and can cause goitrogenic effects due to thiocyanate produced during detoxification[77, 78]. Anhwange[15] and Nwaichi et al.[79] reported on the contents and comparative effects of processing on the cyanide, according to their report, heat treatments reduced cyanide content (approximately 100%) in the tested food crops thereby making them suitable and safer for consumption.

The proximate compositions of aniga and epiti moin indicated that they are carbohydrate and protein rich foods with high moisture and low fat content. The average Nigerian diets have been previously reported to be rich in carbohydrate and moisture but low in fat[80]. The high moisture and carbohydrate contents of these foods are a reflection of the proximate characteristics of the raw materials cocoyam, maize and plantain from which the foods were produced[81-84]. The high moisture content of the food samples coupled with rich carbohydrate energy source and protein makes these foods good source of nutrient for man, however, this also connotes easy microbial proliferation in these foods.

The fact that aniga and epiti moin are rich nutrient sources containing phytochemicals at concentrations beneficial to man is established. They are foods that could be used to augment the nutritional need of man. It is advanced that the application of good manufacturing practices (GMP) and effective hazard analysis critical control (HACCP) in the production of these foods is necessary, to standardize the processing procedures, curtail microbial contamination to levels within standard acceptable limit, and thus optimize the foods for wider consumer acceptability.

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