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Microbiological and chemical compositions of agbarati and ogiri Igbo, popular foods of south eastern Nigerians

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Abstract: The microbial and chemical compositions of two indigenous foods of South eastern Nigeria were investigated. The TAPC of ogiri Igbo ranged from 1.2 x 10^{10} to 2.4 x10^{11}cfu/g, the coliform count was 2.8 x 10^{2} to 7.0 x 10^{6}cfu/g, and fungal count was 1.0 x 10^{8} to 3.3 x 10^{11}cfu/g. Agbarati had a TAPC of 1.1 x 10^{9} to 1.2 x 10^{11}cfu/g, coliform count of 3.9 x 10^{5} to 2.7 x 10^{6}cfu/g, and fungal count of 1.9 x 10^{7} to 2.4 x 10^{7}cfu/g. Species of *Bacillus, Streptococcus, Staphylococcus, Micrococcus, Geotrichum, Mucor, Rhizopus* and *Saccharomyces* were prevalent in the samples. The chemical analysis of the food samples indicated the presence of saponin, tannin, alkaloid, flavonoid, oxalate and cyanide in varied amounts. The nutritional analysis revealed that agbarati and ogiri had moisture contents of 11.40 and 36.12%, Fat contents of 39.10 and 30.24 %, Fiber contents of 2.91 and 3.65%, Protein contents of 29.63 and 12.75%, Ash contents of 4.25 and 1.62% and Carbohydrate contents of 12.71 and 15.62, respectively. These traditional foods are good sources of nutrients however, their microbial loads above 10^{6} tolerant limits and the presence of coliforms calls for concern, adequate hazard analysis and critical control point (HACCP) measure and effective good manufacturing practice (GMP) is imperative in the production of these local foods.

Key words: Indigenous foods, Agbarati, Ogiri Igbo, HACCP, GMP

INTRODUCTION

Food is any substance of animal or plant origin consumed to provide the body with nutrients, which help to build and maintain the body's structure, functions and supplies the energy need of the system[1]. Plants and animals from which foods and nutrients are derived are naturally endowed with

normal microbial flora and chemical substances to protect/defend them from prey and pests. Some of these natural endowments (normal microbial flora and chemical constituents) plus contaminating microorganisms and chemical substances acquired from the environment are pathogenic and toxic to the consumer (man/animals). Some natural chemical substances in plant and animal food products could also be anti-nutrients preventing the absorption of nutrients from food[2, 3]. Food processing is meant not only to improve the taste of the food, improve palatability and digestibility, it also serves to detoxify chemical constituents and destroy/ reduce the microbial load of the food. Contrary to these, processing procedures can also introduce contaminants of pathogenic microorganisms and toxic chemicals. Traditional foods are generally viewed as 'more healthy' from the context of the foods being free from preservative(s) and minimally processed (devoid of the rigorous industrial processing that often removes some nutrients). Fermented and non-fermented traditional foods like ogiri Igbo and agbarati are often processed in the 'traditional ways' by crude implement and fermented by mixed (unclassified and unidentified) microbial flora, some of which could be pathogenic. The chemical compositions (nutritional and anti-nutritional constituents) of some of these foods are equally poorly understood. The foods are generally of diverse quality and of low shelf life due to lack of standards in processing, consequently acceptability is limited to the natives/ indigenes as a culture.

To commercialize Nigerian indigenous foods from different ethnic groups, there is the need for standardization that will make the foods acceptable by other ethnic groups outside their place of origin and by the international community. To this end, the aim of this study is to examine the microbial and chemical compositions of Agarati and Ogiri with a view to creating awareness on the qualities of these foods.

MATERIALS AND METHODS

Description of Samples: Ogiri Igbo is a fermented oily paste produced from castor oil seed (*Ricinus communis*). It serves as a cheap soup condiment among rural dwellers in the south eastern Nigeria. Ogiri is a popular food condiment used to complement or replace magi in soup preparation. It gives a good taste to the soup and also serves as a good source of nutrient and energy. Similar to ogiri Igbo are other ogiri types produced from Melon seeds (*Citrullus vulgaris*), Fluted pumpkin bean (*Telferia occidentalis*) and Sesame seed (*Sessamum indicum*). The production process for all types of ogiri is still a traditional family art and fermentation is by chance inoculation [4, 5].

Agbarati is a traditional food produced in south eastern and south southern parts of Nigeria. It is prepared mainly from mixture of blended melon (*Citrilus vulgaris*) and fungus (sclerotium of *Pleurotus tuber reguim*). The melon-fungus mixture is wrapped in palm frond or banana leaf, tied and cooked to produce a melon-fungus cake (Agbarati). It is often consumed as snacks to pass time especially between meals and as garnish in melon, vegetable or okra soups and other dishes.

Sample collection: The samples were obtained from different parts of Imo State. The ogiri samples were purchased from three different locations – Eziobodo, Ihiagwa and Mbieri. Agbarati was obtained from Anara and Mbieri markets. Samples were placed in sterile specimen containers and transported in cold packs to the laboratory for analysis within one hour of collection.

Sample preparation and enumeration of microbial isolates: Microbial isolation and identification was by standard microbiological techniques. One gram samples were homogenized in nine mL normal saline and serially diluted to 10^{-6}. Aliquot 0.1 mL of appropriate dilutions was spread inoculated in duplicate onto Nutrient agar, MacConkey and Potato Dextrose Agar (PDA). The inocula were spread with sterile spreader to ensure even distributions before incubating the plates. Nutrient Agar and MacConkey Agar were incubated at 37± 2°C for 24 – 48h for the growth of heterotrophic

bacteria and coliforms, while PDA plates were incubated at 28 ± 2°C for 3 to 5 days[6]. Colonies were enumerated at the end of incubation period using digital colony counter (Gallenkamp, England). The isolates were characterized on the bases of colonial morphology, microscopic and biochemical characteristics to include indole production, methyl red, Voges-Proskauer, citrate utilization, motility, spore stain, urease production, catalase, oxidase, coagulase, starch hydrolysis, gelatin liquefaction, fermentation of glucose, lactose, sucrose, maltose, mannitol, xylose, raffinose, arabinose, temperature and salt tolerance tests. Further identification of bacteria isolates was by Biomerieux® sa API kit and with reference to standard identification manuals[7-9].

Determination of chemical compositions of samples: The standard methods of the Association of Official Analytical Chemists[10] were employed in the determination of proximate composition of the samples. The total nitrogen was determined by Micro-Kjedalh method and protein value was derived from the nitrogen content by multiplying by a factor of 6.25. Lipid was by the use of Soxhalet extraction, moisture and ash were determined from oven dried samples and use of muffle furnace respectively, crude fiber was based on weight on incineration while carbohydrate content was assayed by difference[10]. Samples were also analyzed for the presence of alkaloid, cyanide, flavonoid, oxalate, saponin, and tannin following standard methods.

Determination of Alkaloid: Alkaloid was determined following the alkaline precipitation method by Harborne[11]. A measured weight of each processed sample was dispersed in 100ml of 10% acetic acid in ethanol solution. The mixture was shaken vigorously and allowed to stand for 4 hours at room temperature with shaking every 30min. The mixture was filtered through Whatman filter paper (No. 42) and the extract concentrated by evaporation. The extract was treated with concentrated ammonia solution to precipitate the alkaloids. After washing with 1% NH4OH solution, the precipitated alkaloid was dried at 60°C and weighed after cooling, the percentage alkaloid content was calculated. Replicate determination was carried out.

Determination Cyanide: The method as described by Anhwange *et al.*[12] was adopted. Ten gram sample was soaked in 10: 200 v/v orthophosphoric acid in distilled water. The mixture was kept for 12 hours to release all the bonded cyanide. The mixture was then distilled. Aliquot 8 cm^3 of ammonia solution (6 $moldm^{-3}$) and 2 cm^3 of potassium iodide (5%) solution were added to 20 cm^3 of the distillate in conical flask containing 40 cm^3 of distilled water. The mixture was titrated with silver nitrate (0.02 $moldm^{-3}$) to faint but permanent turbidity (1 cm^3 0.02 $moldm^{-3}$ $AgNO_3 \equiv 1.08$ mg HCN). Replicates determination were done for each of the samples [12].

Determination of flavonoid: The method of Harborne[11] was used. Five gram sample was boiled in 100ml of 2MHCl solution for 40min. It was allowed to cool to room temperature before being filtered through Whatman filter paper (No. 42). Flavonoid in the extract was precipitated by drop-wise addition of concentrated ethyl acetate until in excess. Following filtration, the flavonoid precipitate recovered was oven dried and the weight of flavonoid obtained by difference and expressed as a percentage of the sample analyzed.

Determination of Oxalate: The method of Oke [13] as described by [1] was adopted. To 1.0g of sample in volumetric flask was added 190 ml of distilled water and 10 ml of 6M HCl. The mixture was warmed in water bath at 90ºC for 4 h and the digested sample centrifuged for 5 min at 2,000 rpm. Precipitates from the supernatant were filtered off and washed and then titrated with concentrated ammonia solution. The solutions were heated to 90ºC and the oxalate was precipitated with 5% calcium chloride ($CaCl_2$) solution.

Determination of Saponin: The method as described by Obadoni and Ochuko[14] was used for saponin determination. Twenty gram portion of processed sample was dispersed in 200ml of 20% ethanol. The

suspension was heated at 55°C in water bath for 4h with continuous stirring. The mixture was filtered and the residue re-extracted with 200ml of 20% ethanol. The combined extracts were reduced to 40ml over water bath at about 90°C. The concentrate was transferred into a 250ml separatory funnel and 20ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered, and 60ml of n-butanol added. The combined n-butanol extract was washed with 5% aqueous, sodium chloride. The organic layer was separated and evaporated to dryness using a water bath. The samples were dried in the oven to constant weight.

Determination of Tannin: Tannin was determined following the method as described by Sarkiyayi and Agar[1]. To 400 mg sample was added 40 ml diethyl ether containing 1% acetic acid (v/v) the mixtures were mixed to remove pigment materials. The supernatant was carefully discarded after 5 min and 20 ml of 70% aqueous acetone added and the flask sealed with cotton plug covered with aluminum foil, then kept in shaker for 2 h for extraction. The flask content was filtered through Whatman filter paper (No. 42). Aliquot 0.5 ml filtrate was made up to 1.0 ml with distilled water and 0.5 ml Folin Ciocalteu reagent added and then mixed properly before 2.5 ml of 20% sodium carbonate solution was added and further mixed. The mixtures were kept for 40 min at room temperature, after which absorbance was taken using spectrophotometer and concentration was estimated from the tannic acid standard curve.

STATISTICAL ANALYSIS

The microbial counts and chemical compositions 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

Table 1 shows the mean total microbial count of the samples. Ogiri (OGII) exhibited high TAPC value of 2.40×10^{11} $cfug^{-1}$sample, while agbrati (AGM) had the least value of $1.10 \times 10^{9}$$cfug^{-1}$ sample. The coliform count ranges from 2.80×10^{2} to 1.30×10^{9} $cfug^{-1}$ in ogiri, while the fungal count ranges from 2.40×10^{7} in agbarati to 3.30×10^{11} $cfug^{-1}$ samples in ogiri. Table 1 also revealed that ogiri samples are more contaminated when compared to agbarati samples.

Table 1: Mean total microbial counts (cfu/g sample)

Sample code	Total aerobic plate counts	Coliform counts	Fungal counts
OGI	1.20×10^{10}	1.30×10^{6}	1.00×10^{8}
OGM	2.69×10^{10}	2.80×10^{2}	1.15×10^{8}
OGM2	1.86×10^{11}	7.00×10^{6}	1.80×10^{11}
OGII	2.40×10^{11}	9.80×10^{5}	3.30×10^{11}
AGM	1.10×10^{9}	3.90×10^{5}	2.40×10^{7}
AGAN	1.20×10^{11}	2.70×10^{6}	1.90×10^{7}

Key: OG = Ogiri samples; AG= Agbarati samples

Table 2 reveals the microbial isolates from samples examined. The identified isolates are species of *Bacillus, Enterococcus, Micrococcus, Staphylococcus aureus,* and *Escherichia coli*. The fungal isolates included *Rhizopus, S. cerevisiae, Mucor* spp and *Geotrichum. Mucor* spp. was recovered from ogiri.

Table 2: Microbial isolates from samples examined

Food sample	Microbial isolates .
Agbarati	*Bacillus subtilis, Streptococcus spp, Staphylococcus aureus, Micrococcus luteus, Escherichia coli, Geotrichum candidum, Rhizopus stolonifer, Saccharomyces cerevisiae*
Ogiri	*B. subtilis, M. roseus, E. coli, M. luteus, S. aureus, Streptococcus spp, Saccharomyces cerevisiae, Mucor sp., Rhizopus stolonifer, Saccharomyces elipsoideus.*

Tables 3 presents the chemical compositions of the food samples investigated. Both 'agbarati' and 'ogiri' samples had saponin, tannin, alkaloid, flavonoid, oxalate and cyanide. Although the 4.72% tannin and 3.60% flavonoid contents of 'agbarati' are higher than those of 'ogiri', a higher alkaloid and cyanide compositions of 4.70% and 1.39% was found to be present in ogiri. The protein content of 29.63% recorded for agbarati was higher than 12.75% obtained from ogiri. The fat, fibre and carbohydrate compositions of agbarati and ogiri are not significantly different at P= 0.05, however, ogiri had significantly higher moisture and lower ash compared to agbarati.

Table 3: Chemical compositions of Agbarati and Ogiri

Food sample	Nutritional Compositions (%)					
	Moisture	Fat	Fibre	Protein	Ash	Carbohydrate
Agbarati	11.40	39.10	2.91	29.63	4.25	12.71
Ogiri	36.12	30.24	3.65	12.75	1.62	15.62
	Antinutritional composition (Mg / 100g)					
	Saponin	Tanin	Alkaloid	Flavonoid	Oxalate	Cyanide
Agbarati	0.45	4.72	0.54	3.60	0.013	0.05
Ogiri	0.80	0.35	4.70	2.80	0.73	1.39

DISCUSSION

The values of mean microbial counts reported for agbarati and ogiri Igbo analyzed in this work are higher than the value of 1.00 x 10^6 $cfug^{-1}$ heterotrophic contaminants tolerant limit recommended by ICMSF and MGFRF for all components cooked in manufacturing process. The high coliform counts are an indication of poor sanitary practices in the production of these samples[2, 3]. These could be explained to mean that effective quality control of the production processes of these foods are necessary to help reduce/eliminate contaminants, some of which could be pathogenic.

The presence of diverse species of bacteria and fungi in agbarati and ogiri samples corroborate the findings of some other research works that implicated diverse bacterial and fungal species in ready to eat foods and food condiments[15-18]. The presence of bacillus species in agbarati and ogiri Igbo could be explained by the fact that bacillus species are spore bearers that frequently inhabit dust particles, natural water, vegetation, sediments and many foods[19, 20]. Some bacillus species e.g. *B. anthraces*, *B. cereus* are known to be pathogenic, however, majority of bacillus species are food spoilage organisms or opportunistic pathogen. The isolation of *B. subtilis* from agbarati and ogiri may not be a cause for concern because it rarely causes food poisoning[21] and have been used as a probiotic[22] and also involved in the fermentation of ogiri [4, 23]. Similarly micrococci have not been implicated in food borne

infections; these organisms however should not be treated with levity and as mere contaminants because they are known to cause disease in severely immunocompromised patients[24].

The isolation of *E. coli* and *Streptococcus spp* from some of the samples indicates poor sanitary standard post processing. Although some strains of these organisms have been used as probiotic and are known to play a role in food fermentation, they are indicators of faecal contamination and have been implicated in food spoilage. Pathogenic strains of *E. coli* have been implicated in food and water borne infections. Strains of both organisms are associated with nosocomial infections, endocarditis, bacteremia, urinary tract infections (UTI), meningitis, and other infections in humans[21, 25-29].

Staphylococcus aureus in agbarati and ogiri could be from equipment, food contact surfaces or human contamination of product post processing. It is a normal flora of man and some enterotoxin producing strains cause food poisoning specifically when there is temperature-time abuse. *Staphylococcus aureus* is a common cause of skin infections (boils, carbuncle, whitlow, scalded skin syndrome), sinusitis, endocarditis, and bacteremia[30-32].

The isolation of mould species *Rhizopus, Mucor, Geotrichum* from the samples could be explained by the fact that moulds are spore bearers and common environmental contaminants often isolated from food and food products. Rhizopus, *Mucor and Geotrichum* are common saprobic fungi found on a wide variety of organic substrates, they are common agents in fruits spoilage[33]. Some Rhizopus species are opportunistic agents of human zygomycosis[34, 3].

Saccharomyces (sugar fungus) are saprophytic fungi often associated with fermentation and spoilage in sugar rich foods, juice and condiments. Their presence in agbarati and ogiri Igbo could be attributed to fermentation and contamination from the environment[36].

Flavonoid, saponin, tannin, alkaloid, oxalate and cyanide (Phytochemicals) were detected in agbarati and ogiri products. This could be explained because these are foods of plant origin. These phytochemicals are known to have antioxidant activity and are mainly responsible for the medicinal properties of plants and plant products. Flavonoids for example are known to suppress the effects of active oxygen species (H_2O_2 and O_2) in many vulnerable biological systems[37]. Flavonoids are used as natural anti-oxidants in food, medicinal and non-nutritive plant materials due to their ability to inhibit and scavenge reactive oxygen species[38-42]. Flavonoid drugs are used in the management of circulatory disorders involving capillary dysfunction. They were also effective in preventing or alleviating capillary fragility and permeability[43]. Flavonoids are also used to potentiate the *in vivo* and *in vitro* activity of other drugs[44, 45]. Above certain concentrations however, some of these chemicals (Phytochemicals) can serve contrary function as anti-nutrients. Heat processing and fermentation are known to reduce phytochemicals from anti-nutritional concentration levels often present in raw food to acceptable nutritional level in products ready for consumption.

Nwaichi *et al.*[46] reported the comparative effects of processing on the cyanide content of *Manihort esculenta, Glycin max and Zea mays*. They observed that heat treatments reduced the cyanide content (approximately 100%) in the tested food crops thereby making them suitable and safer for consumption.

The proximate analysis of ogiri and agbarati shows they are rich source of nutrient. The higher moisture content of ogiri compared to agbarati could be because ogiri is a fermented product, and fermented products are known to contain high moisture content[47]. The relative high moisture content of the food samples may imply that they are liable to be spoiled by microorganisms[48]; this is because high moisture content has been described to favor bacterial growth[49].

The high fat content of agbarati and ogiri, tend to support the assertion that heating and fermentation enhances the availability of oil content in food products of *Citrullus* spp[6]. In a comparative study on *Citrullus vulgaris, Citrullus colocynthis,* and *Cucumeropsis mannii* for ogiri production, Akinyele and Oloruntoba[6] reported that fat content was lower in unfermented samples than fermented samples. The crude fat content reported for agbarati in this study is comparably higher than those reported for similar other food products from other cultures *kantong* (10.46%) [50], *ekpan nkukwo* (13.20 ± 0.15%)[51] and *mgbam* (36.35 ± 2.32)[48].

The carbohydrate values reported for agbarati and ogiri in this study are low; however, the protein contents of these products are high. This is because, in addition to oil, melon seeds are known for their richness in protein. This is in tandem with the reports of[52] who reported that the seeds of melon are rich in oil and protein. Similarly, Ogbe and George[53] reported that the melon husk contains high level of crude protein. The relatively higher value in nutritional composition reported for agbarati compared to ogiri in this study, could be attributed to the rich nutritional composition of the sclerotium of the fungus *Pleurotus tuber reguim* used in the production of agbarat[48, 54.]

Agbarati and ogiri Igbo are rich nutrient sources that could be used to augment the nutritional need of man. The fermentation process also enriches the products with probiotics. The presence of coliforms however, calls for effective application of hazard analysis critical control point (HACCP) in the processing of these foods.

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