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Microbial Loads of *Ogiri-Ahuekere* Condiment Produced from Groundnut Seed (*Arachis hypogaea* Linn)

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Abstract

Microbial loads of *ogiri-ahuekere* condiment produced from groundnut seeds were examined. The groundnut seeds were sun-dried for 8 hours, dehulled and boiled for 8 hours using kerosene stove. The cooked cotyledons were milled manually into a paste and wrapped in small portions (30g) with blanched plantain leaves. The wrapped samples were fermented in a container for 1-10 day(s) while the unfermented cooked groundnut paste was used as a control. The freshly prepared samples of *ogiri-ahuekere* were used for microbial analysis and this action was carried out under sterile aseptic conditions. Statistical analysis of the data was carried out using ANOVA method with application of SPSS version 20. The significant difference between the mean values was determined by Tukey's test at 95% level of confidence. There was no growth in unfermented sample for TCC and TFMC while there was growth for TBC. There was significant increases in TCC which ranged from` 0.67-6.47 x 10^7 cfu/g, TBC (0.83-8.60 x 10^7 cfu/g) and TFMC (0.30 – 4.90 x 10^7 cfu/g). The results obtained from the study have shown the prevalence of bacteria throughout the period of fermentation in an increasing population.

Keywords: Fermentation, Coliform count, Bacterial count, Fungal count, Colony, Paste.

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Contents

Contribution of this paper to the literature

The study of the microbial loads of *ogiri-ahuekere* condiment showed the contamination of the condiment as well as the number of cells of different microbial counts. *Ogiri-ahuekere* condiment will compete favourably in the market if processed or handled under sanitary conditions.

1. Introduction

The scientific classification of groundnut as kingdom (*plantae*), order (*fabales)*, family (*fabaleae)*, sub-family (*faboideae*), tribe (*aeschynomenae)*, genus (*arachis*) and specie (*hypogaea*) [1]. Groundnut (*Arachis hypogeae* Linn) is an important oil crop of Brazil origin. It is specie in the legumes or "bean" family (*Fabaceae*). It is also widely produced in Guinea Savannah ecological zone of Nigeria. It is cultivated in tropical and warm temperate climate [2]. Groundnut is an important oil crop of Brazilian origin, cultivated in tropical and warm temperate climates. The crop is grown usually as a component of a variety of crop mixtures including sorghum, millet, cowpea and maize [3].

Sahayaraj and Martin [4] reported that groundnut is an important oil seed and cash crop accounting for more than one third (1/3) of the total oil seeds in India. Beside income for farmers, it provides an inexpensive source of high quality dietary protein and oil [5]. Groundnuts are particularly susceptible to contamination during growth and storage. Poor storage of groundnut can lead to an infection by the mould fungus *Aspergillus flavus*, releasing the toxic and highly carcinogenic substance called aflatoxin. The aflatoxin producing mould exists throughout the groundnut growing areas and may produce aflatoxin in the groundnuts when the conditions are favorable to fungal growth [6].

Condiments are edible substances which are added to impart a particular flavor, enhance its flavor, and in some cultures to complement the dish. Many condiments are packaged in single-sachets/packets e.g. mustard and ketchup. They are prepared from both plant and animal materials using processes in which micro-organisms play active roles in the physical, nutritional and sensory modification of the starting materials. The local condiment is an oily paste with strong putrid ammonical odor made from fermented vegetable protein [7]. Condiments are prepared by traditional methods of uncontrolled solid substrate fermentation resulting in extensive hydrolysis of the protein and carbohydrate components. Condiments are used as soup condiments and they generally have strong aroma [8].

Condiments are excellent sources of proteins with essential amino acids, and also contain lipids, carbohydrates, essential fatty acids and vitamins [9]. Many families in West Africa often used fermented condiments as low cost meat substitute. Fermented condiments improve nutritive values of foods as well as sensory properties as taste enhancers; contain antioxidants and neutraceuticals that promote health [10].

Fermented condiments often have a stigma attached to them as they are considered to be food for the poor [11]. Traditional diets in Nigeria often lack variety and consist of large quantities of the staple foods (cassava, yam, maize) with supplements of plantain, cocoyam, rice and beans depending on their availability and seasons [12]. Soups eaten with the staples are essential components of the diet and may contain a variety of seeds, nuts, pulses and leaves [13].

Fermentation is one of the oldest and most economical methods of producing and preserving foods in developing countries [14]. Fermentation remains of interest since they do not require refrigeration during distribution and storage [15]. Apart from increasing the shelf life, and a reduction in the anti-nutritional factors, fermentation markedly improves the digestibility, nutritive value and flavour of the raw seeds [16]. When fermentation is involved in food processing, microorganisms are present. Fermentation is an energy yielding metabolic process which involves the decomposition of substrate in the anaerobic condition. Members of the fermenting organisms are important; for example, *Bacillus spp* in *ogiri* preparation [1, 2].

Fagbemi, et al. [17] described fermentation of condiments as a multi-step process which does not include a formal inoculation step. Bacteria required for the fermentation appear to be incidental to both the raw and processed materials. Indigenous flora is likely carried over from fermentation to fermentation sieves, trays and bags which are respectively used in condiment production. Contamination of spores from the local environment may also contribute to the fermentation micro flora. It is likely that fermentation begins when the cooled, softened, dehulled oil seeds are packaged with leaves or other materials [18]. Changes over the course of 6 days of fermentation period include a pH decrease for the first four days and an increase thereafter [17].

Achi [14] reviewed some important microorganisms found in fermented condiments after different periods of fermentation at optimum conditions. Ogbonna, et al. [19] determined the optimum pH for the growth of these bacteria as 7.5 while the optimum temperature is 33-40°C for 3 days. The methods employed in the manufacture of fermented condiment differ from one region to another because these processes are based on traditional systems. According to local custom, climate conditions and the type of substrates used, specific process variation occur [8]. In general, fermentation takes place under conditions which the producers have found to be favorable for appropriate growth and activity of microorganisms.

This work is aimed at determining the microbial loads of *ogiri-ahuekere* condiment produced from groundnut seed using natural fermentation method. The production of *ogiri-ahuekere* will give value addition to groundnut and as source of income to both the farmers and the producers. This also could help in determining the sanitary condition of the condiment in the market.

2. Materials and Methods

2.1. Collection of Materials

The groundnuts seeds were bought from a local market at Aba, Abia State, Nigeria. Other materials such as swab sticks, syringes, Whatman filter papers No 1, foil, cotton wool, hand gloves, cover lips, distilled water and media produced by Titan Biotech Ltd and Micro-master Ltd, India were purchased from Rufus Chemicals, Aba. Other utensils used were produced by Search tech Ltd, India and they include glass wares, pots, furnace, desiccators, plates, autoclave, DHP 9032 incubators, Bunsen burner, Olympus microscope and colony counter. The reagents used were of analytical grade and they include iodine solution, ethanol, Safaranin solution, sulphuric acid,

methylene blue, starch, glucose, lactose, sucrose, fructose, maltose, boric acid, sodium hydroxide, bromocresol green and methyl red, EDTA, petroleum ether etc. Reagents were produced by BDH Chemicals Ltd, Poole England.

2.2. Production of Ogiri-Ahuekere from Raw Groundnut Seeds

Five hundred grams (500g) groundnut seeds were weighed and spread under the sun for easy removal of the seed coats (hulls). The hulls were removed by rubbing the seeds in-between the palms in accordance with the method described by Chukwu, et al. [1]. The preparation of *ogiri-ahuekere* was prepared according to Chukwu, et al. [1];Chukwu, et al. [2]. Figure 1 shows the flow diagram for the production of *ogiri-ahuekere*.

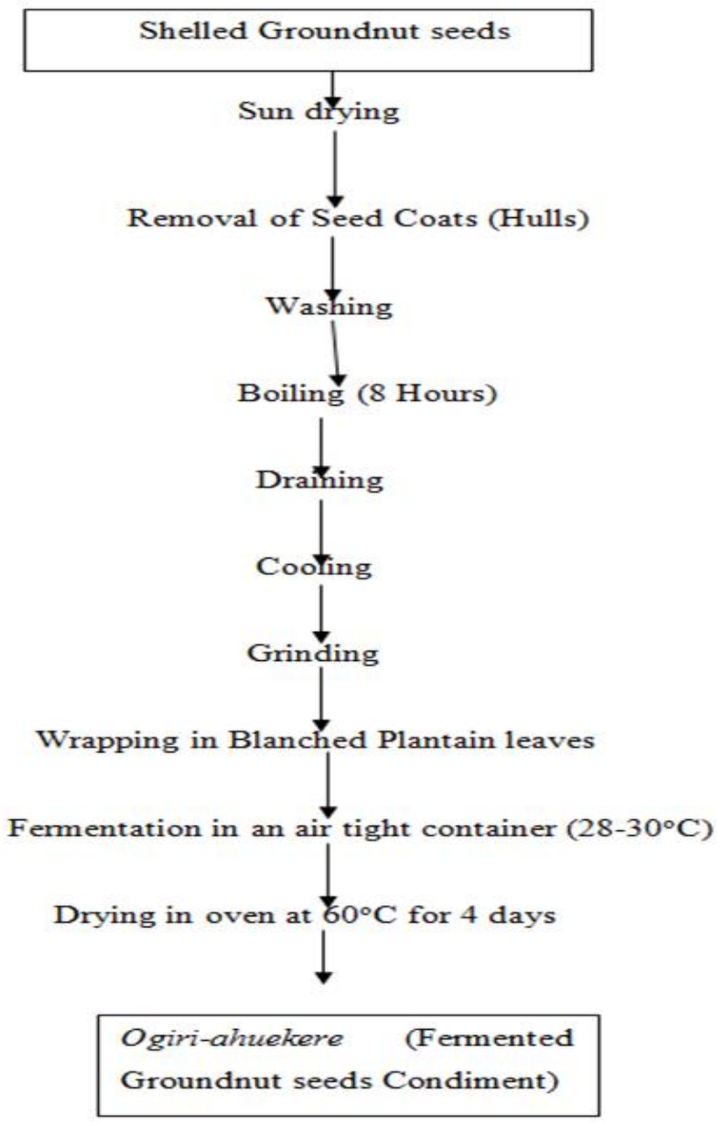

Figure-1. Flow Chart for the Production of Fermented *Ogiri–Ahuekere* Samples.
Source: Chukwu, et al. [8].

2.3. Microbial Analysis of Ogiri-Ahuekere

Freshly fermented samples of *ogiri-ahuekere* were used for microbial analysis

2.4. Media Preparation

The media used were nutrient agar (NA), Salmonella–Shigella agar (SSA), MacConkey agar (MA), mannitol agar, blood agar (BA) and potato–dextrose agar (PDA). PDA was used for fungi isolation while all the other agars were used for bacterial isolations. These agars were prepared as follows: 2.8g of nutrient agar was dissolved in 100ml of water; 11.1g of mannitol was dissolved in 100ml of water; 5.2g of MacConkey agar was dissolved in 100ml of water; 6.3g of SSA was dissolved in 100ml of water; 2.8g of nutrient agar was dissolved in 2ml of blood to form the blood agar; and potato extract was made up to 100ml mark with distilled water. These agars were sterilized in the autoclave at 121°C/15 psi for 15minutes. The media were poured into sterile petri dishes on gelling. These were done in triplicates.

2.5. Serial Dilution

Nine folds serial dilutions were made using 10 test tubes placed on a test tube rack. One gram of the test sample was dissolved in 9ml of normal saline and diluents in a test tube and the content was thoroughly shaken. Subsequent serial dilutions (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}) were made from the solution by adding serially 1ml of the sample solution from proceeding concentration to 9ml of the diluents using sterile syringe. After dilution, 0.1ml of 10^{-5} dilution (10^{5} tubes) was picked out and dropped into different plates of nutrient agar, MacConkey agar, Salmonella–Shigella agar, mannitol agar, and blood agar for bacteria isolation and potato–dextrose agar for fungi

isolation. Both pour plate and streak plate methods were employed. The plates were incubated for 24 hours at 37^{O}C. Plates were examined and the colony growths were counted [20].

2.6. Determination of Microbial Loads of Ogiri-Ahuekere Samples

The method of the ICMSF [21] was employed. One gram (1g) of the prepared sample was dissolved in 9ml sterile distilled water (diluents) and mixed vigorously by shaking. About 1ml of the resultant mixture was aseptically transferred to 9ml sterile water in the test tube and thoroughly mixed. This action was carried out under sterile aseptic conditions. The dilution was continued serially until the sixth dilution was attained. The potato dextrose agar culture plates were incubated at room temperature for two days (48 hours) while the nutrient agar culture plates were incubated at 37^{o}C for 24 hours in the incubator. All the plates were observed daily. The number of colonies formed in each culture plate at the end of incubation period was counted using the Gallenkamp electronic colony method [21]. The total bacterial count was determined by counting the number of viable cells on nutrient agar for bacteria and potato dextrose for fungi plates respectively. Thereafter, the number of viable cells was counted by using model TT201 Colony counter. Total coliform count was determined by introducing the sample inoculums in Eosin methylene blue agar using pour plate method. The plates were incubated aerobically for 18-24 hours at 57^{o}C. The total viable cells from primary plates were calculated using model TT201 Colony Counter. Thereafter, a standard formula was used to calculate the number of the total coliform count [22]. The total microbial counts (cfu per gram) of food sample were calculated according to Equation 1.

$$TMC = \frac{Number\ of\ colonies\ observed \times inoculum\ plated}{Weight\ of\ inoculum \times dilution\ factor} \quad (1)$$

$$TMC = \frac{Total\ viable\ cells \times inoculum\ plated}{Weight\ of\ inoculum \times dilution\ factor} \quad (2)$$

3. Results and Discussion

Table 1 showed the average total microbial counts of *ogiri-ahuekere* produced from groundnut seeds at various fermentation time. The microbial counts included total coliform count, total bacteria count and total fungi/mould count, no growth in the unfermented *ahuekere* sample.

3.1. Total Coliform Count (TCC)

The TCC of unfermented *ahuekere* (0.00) was not significantly ($p \leq 0.05$) different from TCC of fermented *ogiri-ahuekere* sample from 1 day fermentation (0.67×10^7cfu/g). Table showed that TCC of fermented *ogiri-ahuekere* samples from 1 and 2 day(s) were significantly the same while TCC of 2 days fermented *ogiri-ahuekere* sample (1.03×10^7cfu/g) was significantly different from TCC of unfermented *ahuekere* though similar to TCC (1.60×10^7cfu/g) of 3 days fermented *ogiri-ahuekere* sample. It was observed that the TCC (2.43×10^7cfu/g) of 5 days fermented *ogiri-ahuekere* sample was not significantly different from the TCC of 3, 4 and 6 days fermented *ogiri-ahuekere* samples (1.60, 1.93, and 3.20×10^7cfu/g respectively) while the TCC of 6 days fermented *ogiri-ahuekere* sample was significantly different of that of 4 days fermented *ogiri-ahuekere* sample. The total coliform count of 8 days fermented *ogiri-ahuekere* sample was 4.67×10^7cfu/g. The total coliform count (TCC) of 8 days fermented *ogiri-ahuekere* sample was significantly different from TCC of 6 and 9 days (5.70×10^7cfu/g) fermented *ogiri-ahuekere* samples, and it was also the same with TCC of 7 days fermented *ogiri-ahuekere* sample (3.87×10^7cfu/g); although, TCC of 6 and 7 days fermentation were significantly the same. However, the TCC of 9 and 10 days fermented *ogiri-ahuekere* samples were the same statistically but significantly different from the TCC from other samples. The table also illustrated how the total coliform count increased with the fermentation day [23].

3.2. Total Bacteria Count (TBC)

There was also significant increase in the TBC with respect to the days of fermentation. The unfermented *ahuekere* sample had TBC (0.83 x 10^7 cfu/g) which was statistically the same with the TBC from 1 day fermented *ogiri-ahuekere* sample. The TBC (1.37, 1.70 and 2.33×10^7cfu/g respectively) from samples fermented for 1, 2 and 3 days were significantly ($p \leq 0.05$) similar but were significantly different from the TBC (2.80×10^7cfu/g) of 4 days fermented *ogiri-ahuekere* sample. It was observed that after 4 days fermentation, the TBC of the samples increased significantly throughout the period of fermentation Table 1

The TBC of 5 days fermented ogiri-ahuekere sample was 3.47×10^7cfu/g which is significantly different from the TBC (4.40×10^7cfu/g) of 6 days ogiri-ahuekere samples. There were significant difference among the TBC of 5, 6, 7, 8, 9 and 10 days *ogiri-ahuekere* samples. This significant increase in TBC was due to the increase in fermentation time and similar results were reported by Peter-Ikechukwu, et al. [24].

3.3. Total Fungi/Mould Counts (TFMC)

Total Fungi/Mould Counts (TFMC) was also shown in Table 1. The TFMC of all the *ogiri-ahuekere* samples increased significantly with increase in fermentation time. The unfermented *ahuekere* had no fungi growth. The following samples were statistically similar in TFMC: unfermented, 1 and 2 day(s) samples (0.00, 0.30 and 0.60×10^7cfu/g respectively); 3 and 4 days samples (1.00 and 1.33×10^7cfu/g respectively); 6 and 7 days samples (2.73 and 3.23×10^7cfu/g respectively); and 7 and 8 (3.23 and 3.33×10^7cfu/g respectively) days fermented samples respectively. However, the TFMC (2.03, 4.33 and 4.90×10^7cfu/g respectively) from samples fermented for 5, 9 and 10 days were significantly ($p \leq 0.05$) different from TFMC of the rest of the samples. Sanni, et al. [25] and reported that some Nigerian soup condiments have the microbial load within the range of 10^7 and 10^9 cfu/g as well as Peter-Ikechukwu, et al. [23].

Table-1. Mean Microbial Count of *Ogiri-Ahuekere* Fermented for 0-10 Days.

Fermentation time (day)	Total Count (x10^{7}cfu/g)		
	TCC	TBC	TFMC
0	0.00^{a}	0.83^{a}	0.00^{a}
1	0.67^{ab}	1.37^{ab}	0.30^{a}
2	1.03^{bc}	1.70^{bc}	0.60^{ab}
3	1.60^{cd}	2.33^{cd}	1.00^{bc}
4	1.93^{d}	2.80^{de}	1.33^{c}
5	2.43^{de}	3.47^{e}	2.03^{d}
6	3.20^{ef}	4.40^{f}	2.73^{e}
7	3.87^{fg}	5.50^{g}	3.23^{ef}
8	4.67^{g}	6.50^{h}	3.33^{f}
9	5.70^{h}	7.30^{i}	4.33^{g}
10	6.47^{h}	8.60^{j}	4.90^{h}
LSD	1.014	0.843	0.531

Note: Means with the same superscripts are statistically the same but means with the different superscripts are significantly different from each other ($p \geq 0.05$) in the column.
Where
TCC = Total Coliform Counts
TBC = Total Bacteria Counts
TFMC = Total Fungi/Mould Counts
Note: The microbial load increased significantly from 2-10 days fermentation of groundnut seeds.

4. Conclusion

The results obtained from the study have shown the prevalence of bacteria throughout the period of fermentation in an increasing population. Total counts of the microorganisms involved in fermentation process showed that contamination might have occurred either from processing equipment, the handlers or from the raw materials and the environment. Fermentation time caused significant increase in the microbial loads of *ogiri-ahuekere* samples

5. Recommendation

Fermented groundnut condiments could improve nutritive values of foods as well as sensory properties as taste enhancers; contain antioxidants and neutraceuticals that promote health; however it is recommended that further study on the toxicology of this product be carried out before it can wholly be accepted for human consumption.

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