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Effect of packaging on the microbial and physicochemical composition of UGBA (*Macrophylla Pentaclethra Benth*)

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Abstract

The concept of packaging dates back to the early times where traditional methods were used. Packaging material such as plantain leaves, polyethene bags and other variety of local leaves are used to package the fermented ugba (macrophylla pentaclethra Benth) seeds. Fermentation impacts various desirable qualities on the organoleptic properties of ugba. The production process involves boiling of seeds, dehulling, slicing and the final process of natural fermentation. The ugba samples used for this study was obtained from various markets in Port Harcourt metropolly, samples were accessed for microbial contamination by culturing, serial dilution, characterization of isolates, biochemical testing for identification and physicochemical parameters such as proximate composition, pH and temperature were accessed. The toal heterotrophic count of the samples from the different markets showed a high count of 2.57×10^{8}cfu/g (Choba market) to 2.67×10^{8}cfu/g recorded for the leaf wrapped compared to that of nylon wrapped with the least count of 1.92×10^{8}cfu/g. the total fungi count also yielded the highest value for the leaf wrapped with counts of 5.15×10^{4}cfu/g to 9.7×10^{4}cfu/g. All these counts tend to be higher than the microbial standard specified by W.H.O. which states that microbial counts for fermented foods should not exceed 5.0×10^{5} colonies per gram. Microorganisms isolated were Pseudomonas eruginosa, Staphylococcusaureus, Lactobaccillus, sp, Proteus sp, Escherichia coli, Baccillus sp, Micrococcus sp, and Salmonella. Fungal isolates include Saccharomyces sp, Aspergillus niger, Aspergillus flavus, penicillium sp, Candida sp, and Fusarium. The need for consumption of food product safe from microbial contamination cannot be over emphasized. Packaging materials which encourage less microbial contamination should be encouraged, and production and packaging processes should be evaluated.

Keywords: Packaging, microbial, physicochemical.

Introduction

There are various plant seeds that are fermented and used as food in some rural and urban part of Nigeria among which is "Ugba" from African oil bean seed (*Marcophylla pentaclethra* Benth). A woody plant predominant in the rain forest. African oil bean seed locally called "Ugba" or "Ukpaka" by the Igbo communities in Nigeria is a fermented and popular food condiment used as flavoring[1,2] as snack, condiment etc. Fermentation is the technique of biological conversion of complex substrates into simple compounds by various microorganisms such as bacteria and fungi[3]. Food fermentation has over the years become a part of the cultural and traditional norm among the indigenous communities in Africa. Several microorganisms are implicated in the fermentation of ugba as it is a natural fermentation process, predominantly. *Bacillus* specie plays a major role in the fermentation process, *Micrococcus* and *Lactobaccillus* are also implicated.

Packaging is an art, science and technology of enclosing or protecting a product for distribution, storage, sale and use. Packaging materials help in defining the quality of the product.[4] Ugba is not an exception in this process as it is packaged in various ways after production, ranging from local leaves to polythene bags. Leaves play a vital role in packaging of food products. For ugba, leaves such as cocoyam leaves (*Xanthosoma saggitifolium*), and plantain leaves (*Musa paradisiaca*), Okpapia leaves (*Alchornea laxiflora Benth*) are used. Fermentation makes food palatable by enhancing its organoleptic properties; Aroma, texture, taste and flavour [5], of which Ugba is not an exception as the safety of ugba for consumption is directly affected by the packaging materials, storage conditions and processing.

Microbial contamination associated with ugba are; *Pseudomonas, Syaphylococcus*, *Enterobacter*, *Leuconostoc*, *Corynebacteria*, *Proteus app sp*, *Escherichia coli*, *Klebsiella* and *Alkaligenes*[6,7]. Fungal isolates include *Aspergillus* sp, *Penicillium* sp *and Saccharomyces* sp.Ugba is rich in various nutrients such as protein, fats and carbohydrates.[8] African oil bean seed has been known to be a good source of edible protein and high energy calories. It is rich in minerals (Calcium, Phosphorus etc.) needed for body functions such as bone formation, blood coagulation, nervous co-ordination and muscle activities[2].

African oil bean seed (*Macrophylla pentaclethra Benth*) popularly called "ugba" in the Eastern part of Nigeria, is highly consumed by several African communities as a flavoring[1]. Various attributes of this local seed such as its richness in vitamins and minerals, typical aroma and flavor, availability and cheapness makes it desirable and highly consumed. The lack of GMP (Good manufacturing processes) employed in the fermentation process, packaging, storage and retail makes it vulnerable to microbial contamination by pathogenic microorganisms capable of posing serious health risks when consumed as well as the various effects of packaging materials used. This study seeks to evaluate the effects of different retail packaging materials used on the microbial profile and the proximate composition, thus identifying the more preferred packaging material.

The main aim of this study is to evaluate different packaging materials used in wrapping ugba for sale and the effects they have on the microbial profile and physicochemical composition of ugba. Some packaging materials encourages high microbial contamination and dominance while others help to reduce this contamination, some extend the shelf life of the product, while others directly or indirectly encourage deterioration. Ugba which is popularly wrapped in local leaves or polythene bags are to be evaluated for the determination of their safety for consumption.

Materials and methods

Area of study: The ugba used for this study were purchased from four different major markets in Port Harcourt municipality. The various samples were purchased from four different sellers in a market, Rumuokoro, Rumuosi, Alakahia and Choba markets making a total of 16 samples obtained, the nylon wrapped and leave wrapped. These samples were transported to the laboratory in Ziploc bags and sampled within 24hrs.

Sample Collection: A total of 16 ugba samples used for this research were purchased randomly from different markets (Choba, Rumukoro, Alakahia and Rumuosi markets). The samples were collected aseptically into sterile polyethylene bags and were transported to the laboratory for analysis within two hours of collection to maintain the market conditions.

Microbial analysis of the samples: Ten (10) grams of each ugba samples were weighed into a beaker containing 90 ml of sterile peptone water and allowed to stand for 5 minutes with occasional stirring and observation using a sterile glass rod., ten-fold serial dilution was carried out by transferring 1 ml from the supernatant into 9 ml sterile diluents measured in the test tube making dilution 10^{-2}, this was repeated into 9ml diluents to obtain dilution 10^{-3}, till dilution 10^{-5} was obtained. 0.1ml 10^{-4}-10^{-5} of each dilution were dispersed into the Plate count agar in duplicates while 0.1ml of 10^{-2}-10^{-3} into the selective media.

From the aliquot, 0.1 ml was plated onto nutrient agar using spread plate method for total viable heterotrophic count, mannitol salt agar for *Staphylococcus* count, MacConkey agar for coliform count and the plates were incubated at 37ºC for 24hrs. For total fungi count, 0.1 ml of the diluent was plated onto potato dextrose agar (PDA) and incubated at 26ºC for 2-5 days.

Single colonies of bacteria were randomly picked from the different bacteriological media previously incubated based on their morphological characteristics and were sub cultured on freshly prepared media and incubated for 24hrs at 37ºC to obtain a pure culture.

After the purification of isolates, nutrient agar slants were prepared in bijou bottles by dispensing 10 ml of the nutrient agar into bijou bottles and autoclaved at 121ºC for 15 min at 15 psi, slanted and allowed to solidify. After which a sterile wire loop as used to pick each inoculum and streaked onto the surface of the slanted nutrient agar and stabbed into the butt and incubated at 37ºC for 24hours.

Isolates were identified based on their morphological and cultural characteristics on growth media which includes colony size, colour, opacity, consistency, colony pigmentation, elevation, odour, swarming), identification materials, reagents[9].

This was done based on the colonial morphology (colour, size, texture) and the cell morphology (mycelia, hyphae) of the fungi was assesed using lactophenol blue. A piece of the mycelium from the Petri dishes was mounted on a clean grease free slide using a sterile wire loop and covered with a cover slip and a drop of lactophenol cotton blue stain was added and allowed for few minutes before it was examined with the microscope.

Physicochemical analysis: pH: pH of the suspension was determined using a pH metre which was equipped with a reference glass electrode, it was inserted into distilled water to reach pH constant It was then inserted into the ugba homogenate solution and pH were recorded[10].

Temperature: The thermometer was inserted into the ugba homogenate solution of 10g into 10ml peptone water and the temperature of each set was recorded.

Proximate analysis: This was done as described by[11].

Results and discussion

From the study it is observed that ugba samples both (both leaf and nylon wrapped) harbor a wide variety of bacterial and fungal contaminants. The Total heterotrophic counts of the samples indicates that the leaf wrapped ugba samples from the four market had the highest Total heterotrophic bacteria counts which ranged from 2.57×10^{8}cfu/g (Choba market) to 2.67×10^{8}cfu/g (Rumuosi market) than that of the nylon wrapped which ranged from 1.92×10^{8}cfu/g (Choba market) to 2.35×10^{8}cfu/g (Rumuosi market). These findings tend to be higher than the heterotrophic bacteria count of ugba obtained by [12] who reported highest counts of $3.7\pm0.50\times10^{4}$cfu/g.

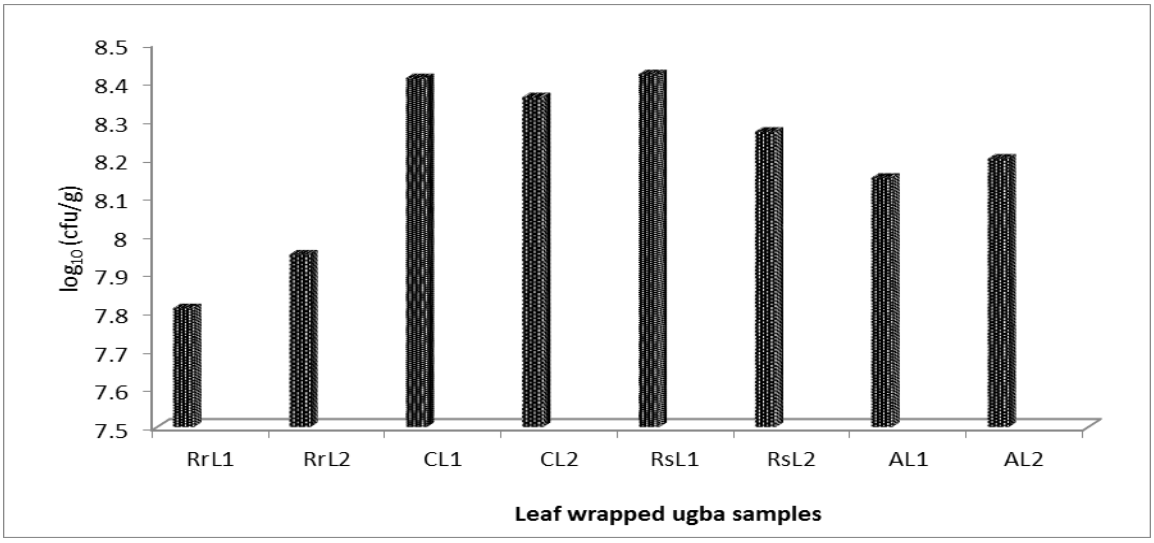

Figure-1: Total heterotrophic counts of leaf wrapped ugba samples obtained different market.

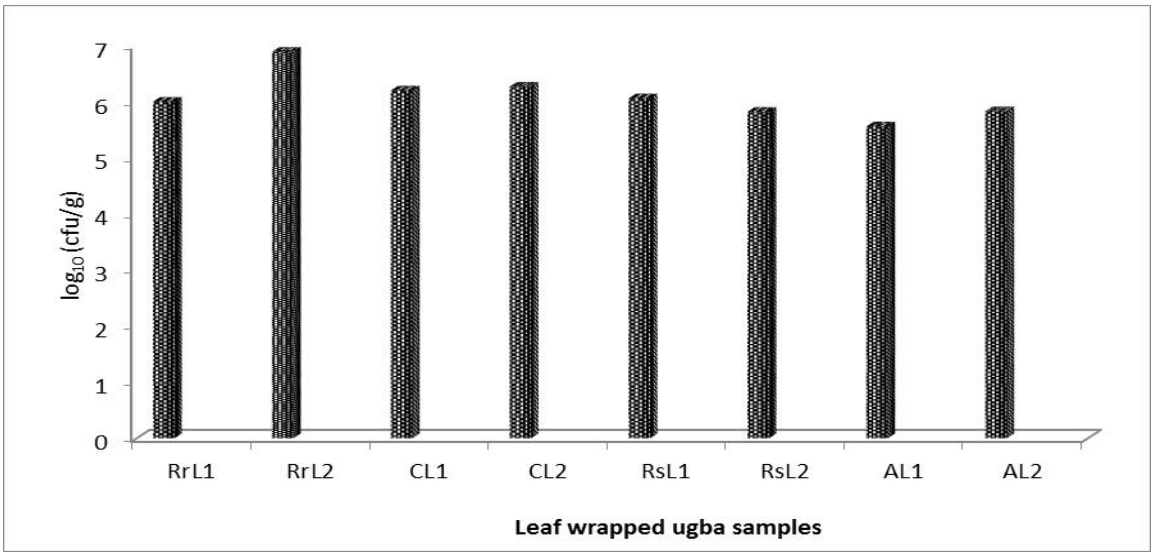

Figure-2: Total *Staphylococcus* counts of leaf wrapped ugba samples obtained different market.

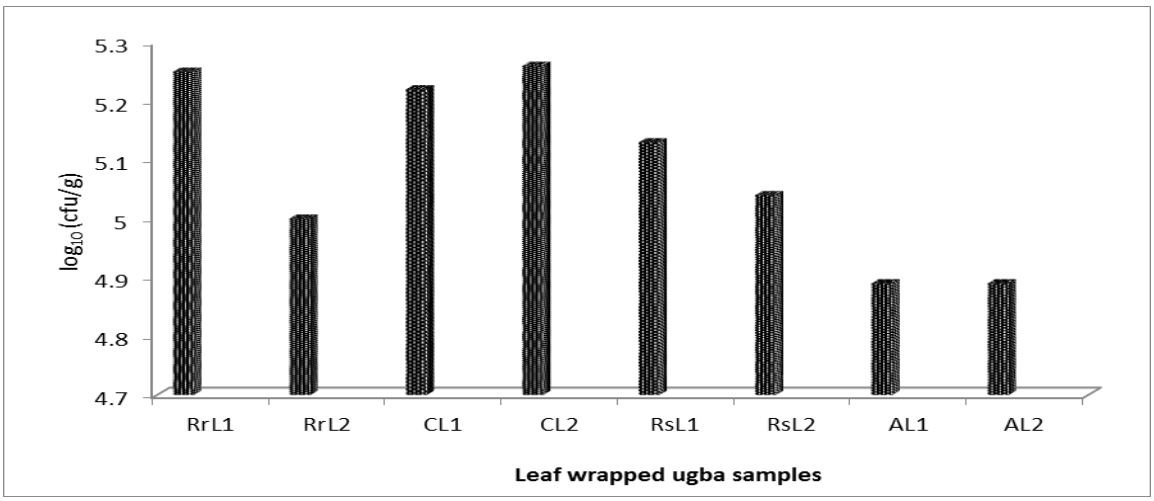

Figure-3: Total coliform counts of leaf wrapped ugba samples obtained different market.

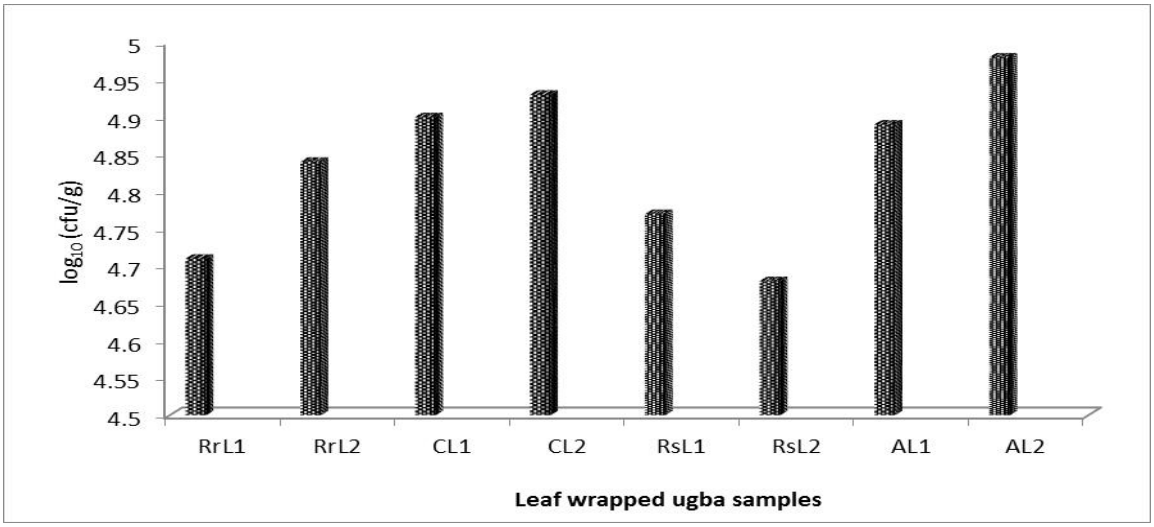

Figure-4: Total fungal counts of leaf wrapped ugba samples obtained different market.

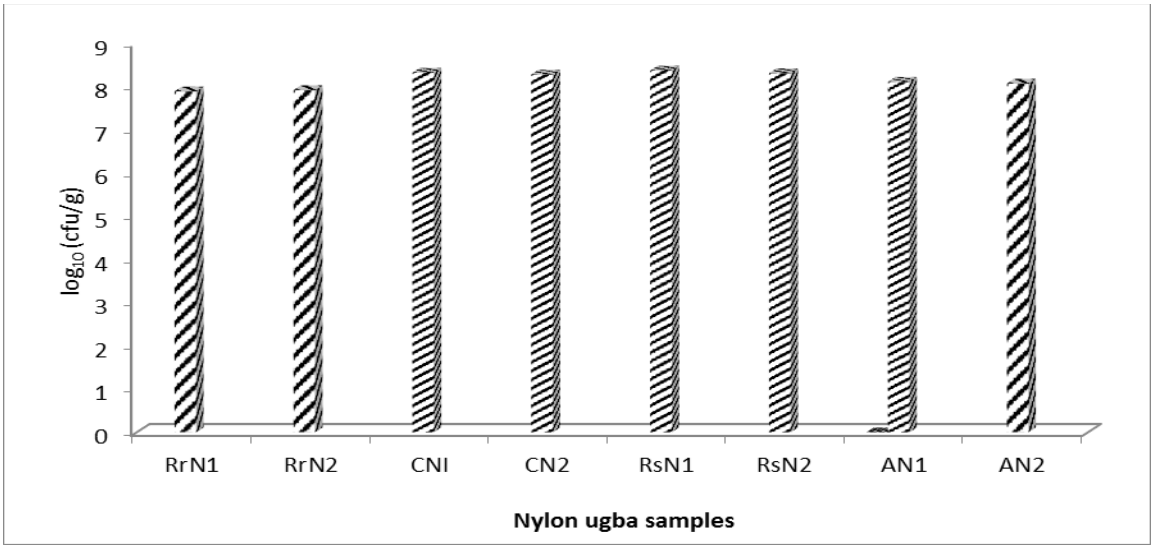

Figure-5: Total heterotrophic counts of nylon ugba samples obtained different markets.

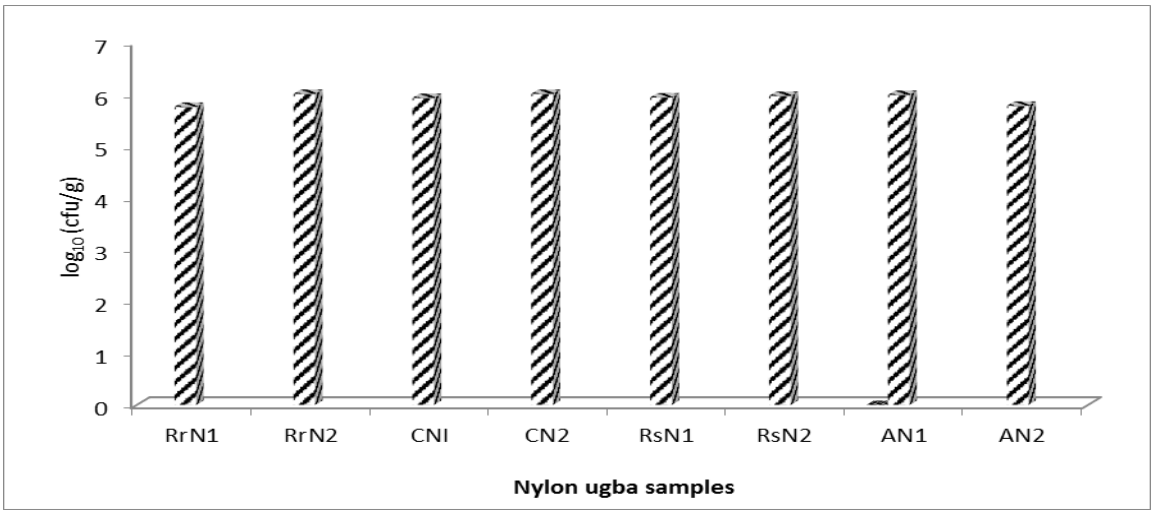

Figure-6: Total *Staphylococcus* counts of nylon ugba samples obtained from different markets

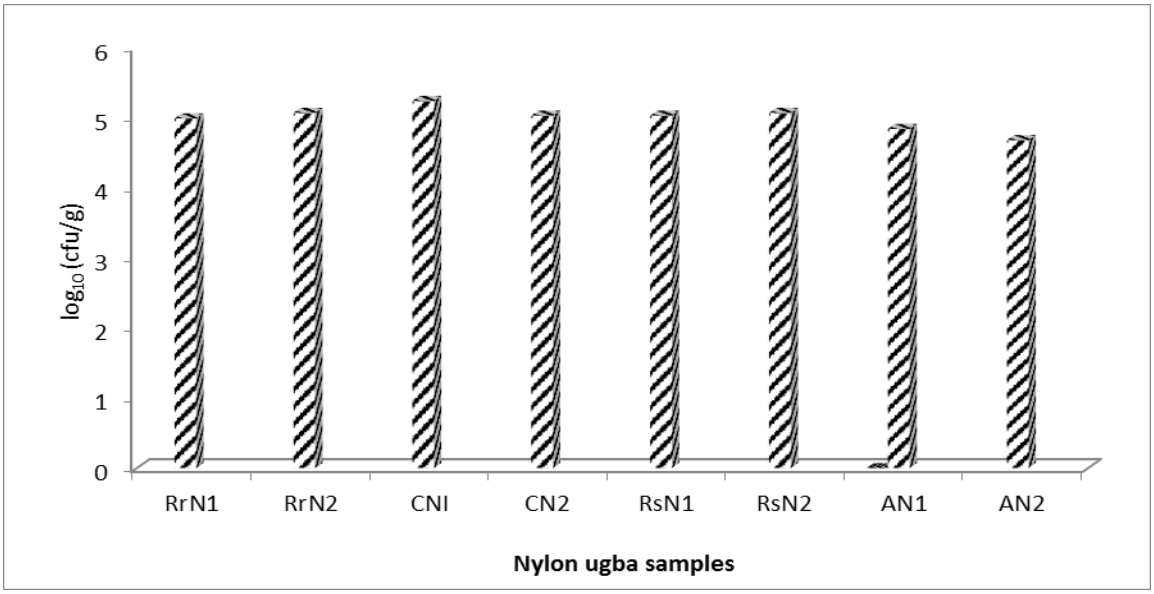

Figure-7: Total coliform counts of nylon ugba samples obtained from different markets.

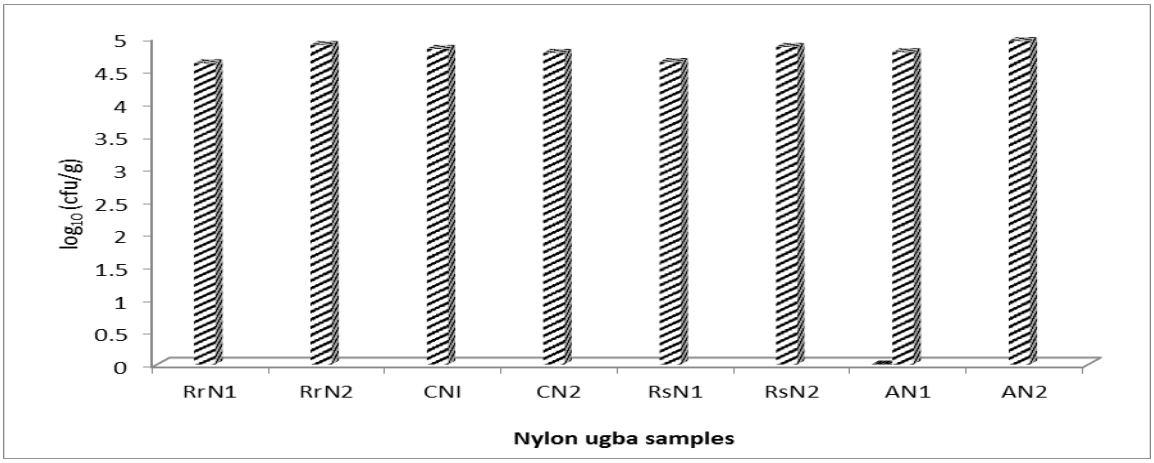

Figure-8: Total fungal counts of nylon ugba samples obtained different markets.

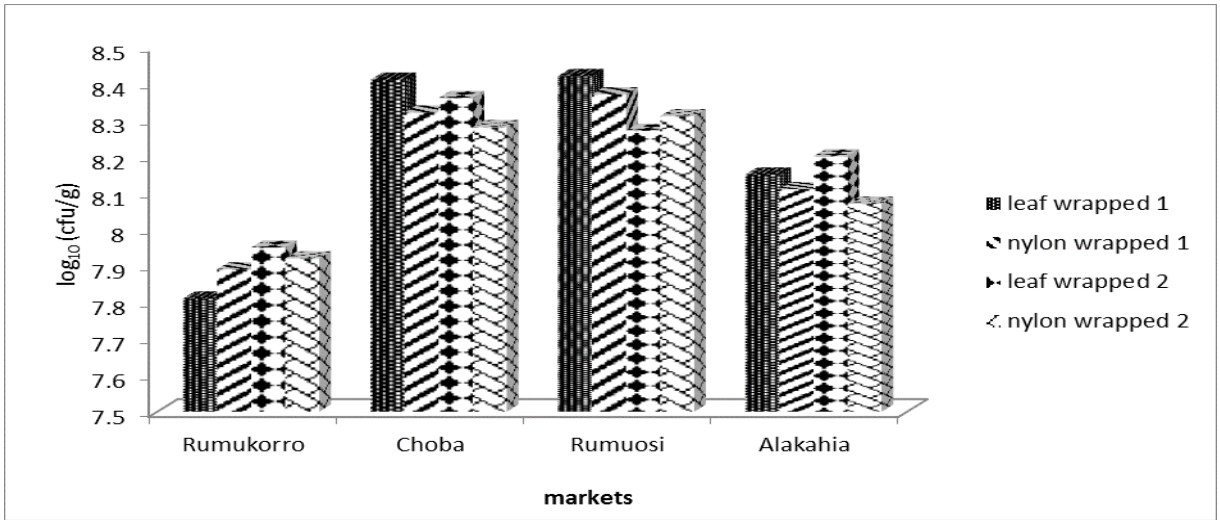

Figure-9: Total heterotrophic counts of leaf and nylon ugba samples obtained from different markets.

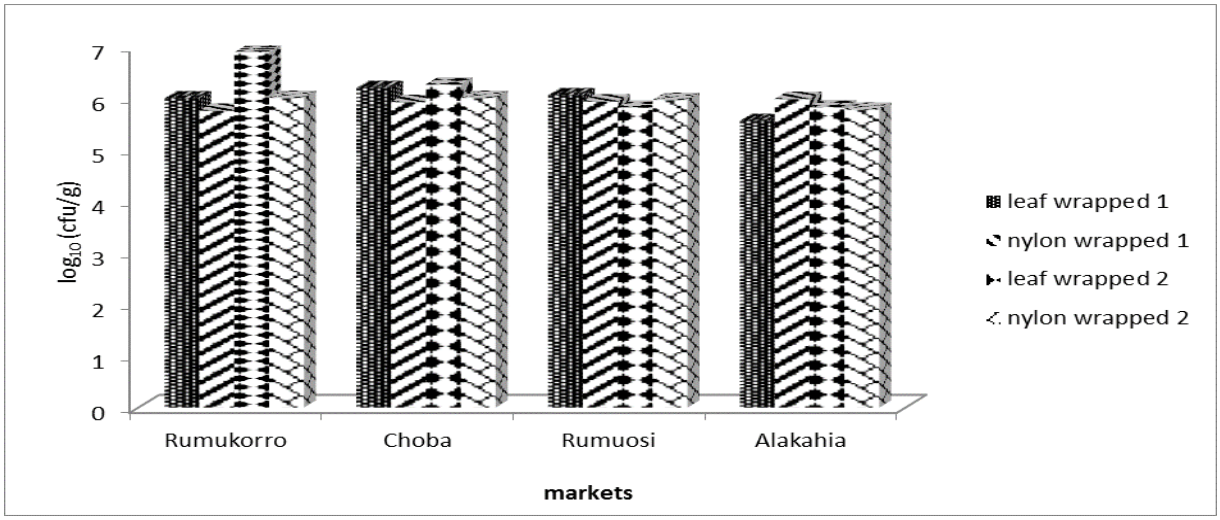

Figure-10: Total *Staphylococcus* counts of leaf and nylon ugba samples obtained from different markets.

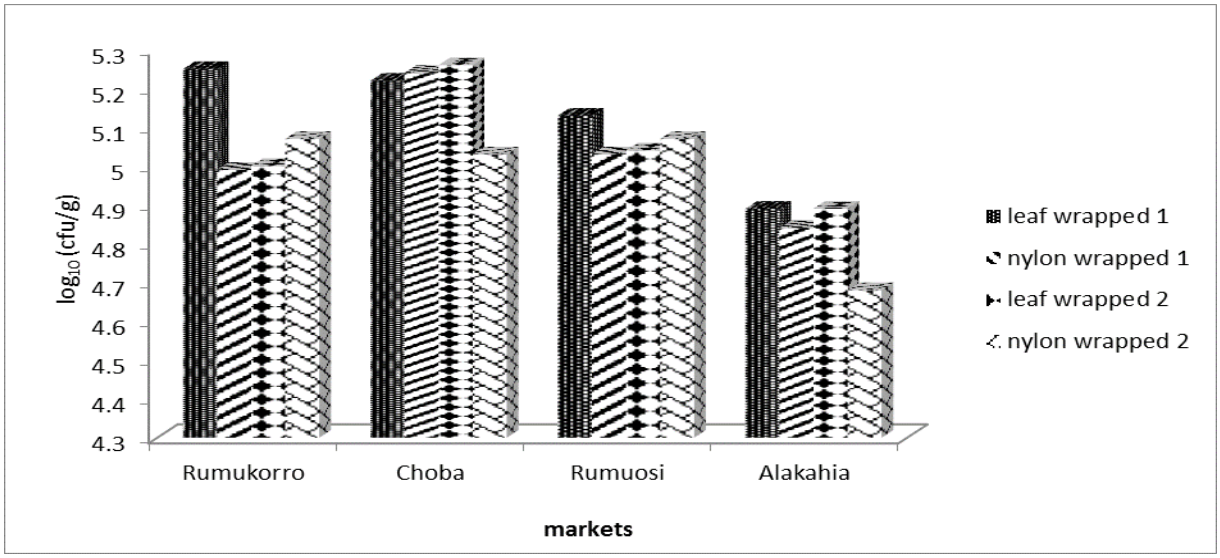

Figure-11: Total coliform counts of leaf and nylon ugba samples obtained from different markets.

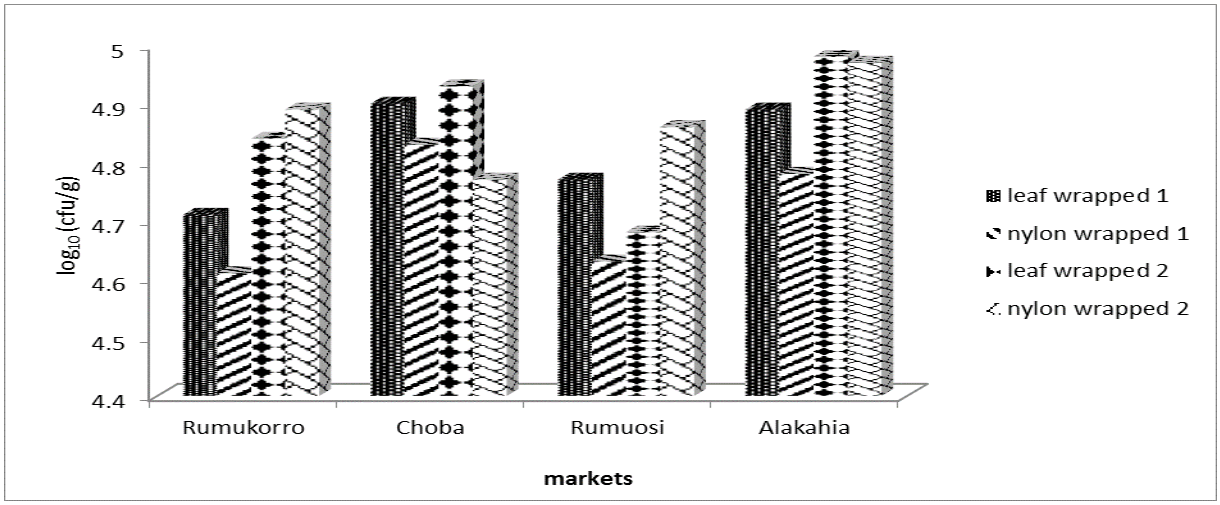

Figure-12: Total fungal counts of leaf and nylon ugba samples obtained from different market.

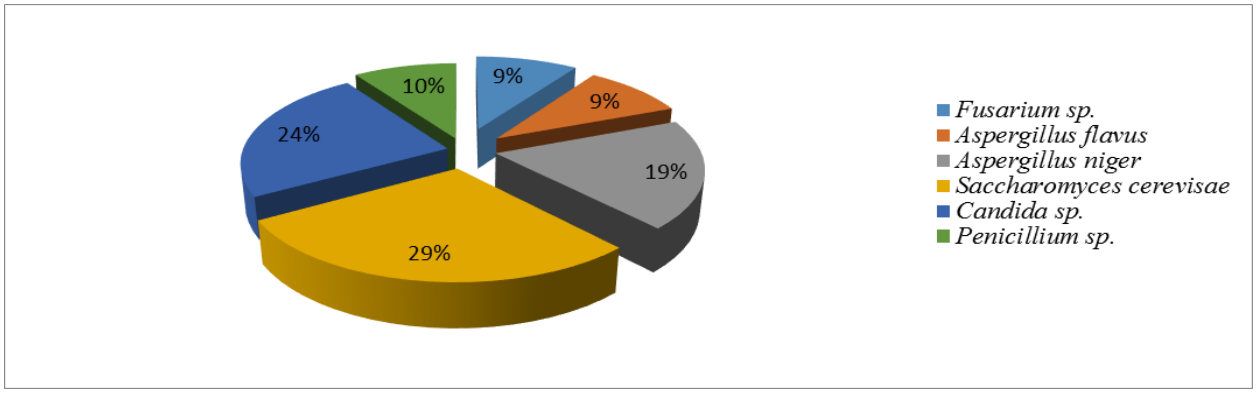

Figure-13: Pie chart showing the most occurring fungi isolated from the nylon and leaf wrapped ugba from different market and their percentages.

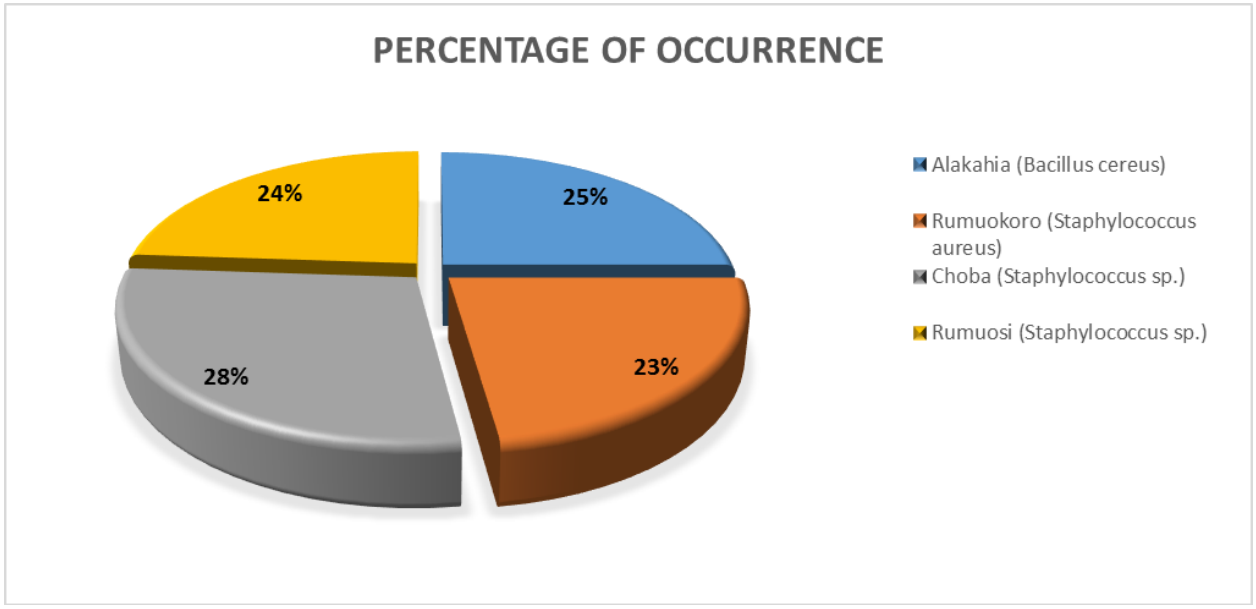

Figure-14: Pie chart of the highest occurring microorganisms isolated from all the samples.

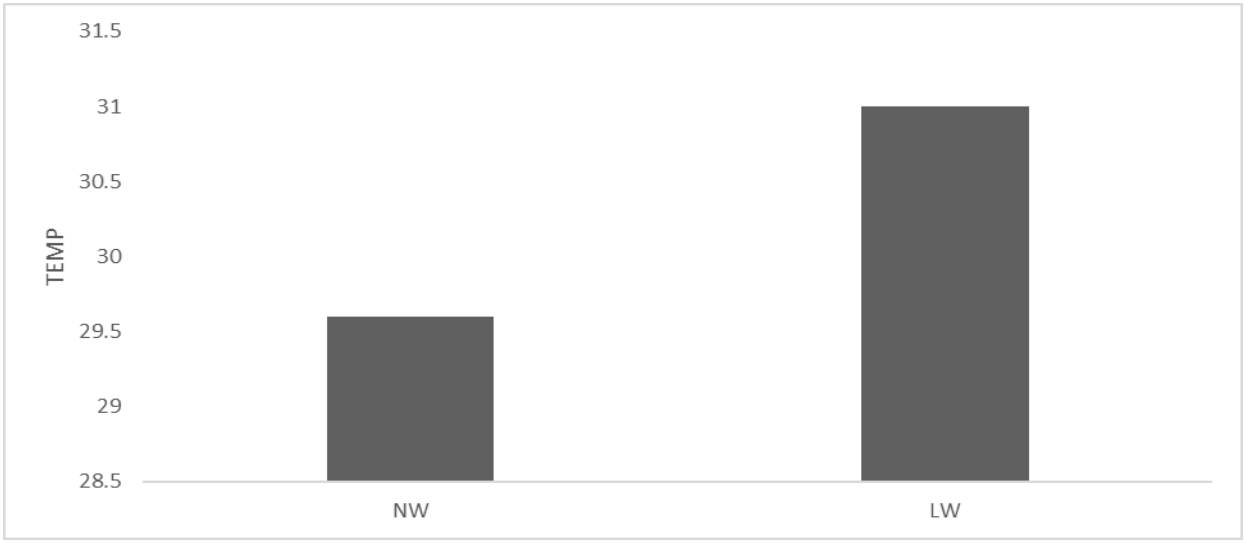

Figure-15: Temperature of the nylon and leaf wrapped ugba from different market.

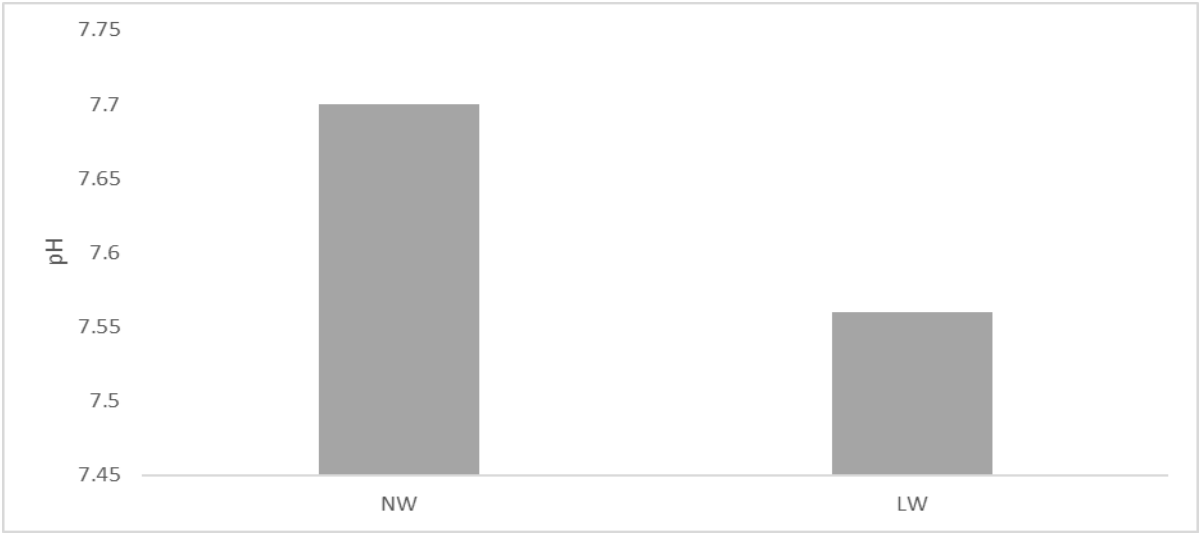

Figure-16: pH of the nylon and leaf wrapped ugba from different market.

Table-1: Proximate analysis of UGBA samples.

Parameters (%)	Nylon wrapped	Leaf wrapped
Crude protein	29.30	15.51
Ash	3.23	6.16
Crude fibre	1.58	7.22
Moisture content	50.34	35.45
Lipid	21.18	19.85
Carbohydrate	3.45	16.08

According to the WHO (World Health Organization) standards for microbial limit of Total heterotrophic bacteria count (THBC) for fermented foods which states that THBC should not exceed 5.0×10^5 colonies per gram of sample and coliform count which should not exceed 5.0×10^3 colonies per gram of sample. The findings from this study tends to be higher than the specified limits while some values were within the limits. In terms of the total coliform counts, highest value of 1.35×10^5cfu/g (Rumuosi) market and lowest count of 7.9×10^4cfu/g (Alakahia market) were obtained for the leaf wrapped and 1.77×10^5cfu/g (Choba market) counts for the highest value and 7.0×10^4cfu/g (Alakahia) for the lowest counts which is slightly higher than the specified limit.

The findings from this study shows a higher coliform counts than the specified microbial limit with few of the counts occurring within limits and thus requires the attention of appropriate National Agencies to inspect the manufacturing processes of locally fermented food products.

The total *staphylococcus* count, leaf wrapped had the highest counts which ranged from 1.89×10^6cfu/g (Choba market) to 7.85×10^6cfu/g (Rumuokoro market) and the lowest counts ranging from 3.65×10^5cfu/g (Alakahia market) to 6.85×10^5cfu/g (Alakahia market) compared to the nylon wrapped with a high value ranging from 1.02×10^6cfu/g (Rumuokoro market) to 1.04×10^6cfu/g (Choba market) and lowest value of 5.8×10^5cfu/g (Rumuosi market) to 9.35×10^5cfu/g (Rumuosi market). The Total *staphylococcus* counts (TSC) from these findings tend to be lower than the counts obtained by [13]who reported $7.89\pm0.14\times10^5$cfu/g.

The high *Staphylococcus counts* obtained from this study was introduced probably by humans during the production process as they are normal flora of the human skin. From the counts obtained from the different markets it is observed that the leaf wrapped has the highest counts which ranged from 2.57×10^8cfu/g to 9.7×10^4cfu/g

Based on the microbial standards for Total Staphylococcus Count by the international commission on microbiological specification four food (ICMSF) which States that *staphylococcus* count for fermented foods $>10^4$cfu/g is unsatisfactory. The counts from these findings both for the nylon wrapped and the leaf wrapped exceeded the stated limits thus requires attention from the national food agencies to evaluate GMP (good manufacturing processes) as related to fermented food products.

The Total fungal counts (TFC) for the four markets yielded a highest count accorded to the leaf wrapped which ranged from 5.15×10^4cfu/g (Rumuokoro market) to 9.7×10^4cfu/g (Alakahia market) compared to that of the nylon wrapped which ranged from 4.1×10^4cfu/g to 8.95×10^4cfu/g as represented in Table-7. According to ICMSF, ready to eat foods with TFC of $\leq10^3$cfu/g are acceptable, 10^4cfu/g-10^5cfu/g are tolerable and $\geq 10^6$cfu/g are unacceptable (ICMSF, 1996)13. Based on this, the total fungal count obtained from this study are of a tolerable range although slightly higher than the counts obtained by (Eze *et al.*, 2014) which ranged from 5.6×10^3cfu/g to 8.5×10^3cfu/g.

The fungal counts obtained from this study was believed to be contamination resulting from the local method of fermentation used in processing of the ugba and disposition of air born fungal spores which multiply if given the right conditions. The microorganisms isolated from this study include *Bacillus sp*., *Pseudomonas aeruginosa*, *Staphylococcus sp*., *Staphylococcus aureus*, *Lactobacillus sp*., *Streptococcus* sp., *Micrococcus* sp., *Proteus* sp. Food pathogens such as *Clostridium perfringes*, C. *botulinum*, *Salmonella sp*.. This concurs with previous studies by[14] [,15]. Although bacteria like *Escherichia coli* and *Staphylococcus aureus* were isolated.

Bacillus sp. and *Lactobacillus sp* obtained in this study are fermentation agents[6,15,16;] This may explain their presence in this study. The percentage frequencies of bacterial isolated from Rumukoro market include *Salmonella* sp. (5%), *Escherichia coli* (5%), *Lactobacillus sp*. (5%), *Staphylococcus aureus* (10%), *Proteus sp*. (10%), *Staphylococcus sp*. (11%), *Bacillus sp*. and *Pseudomonas aueroginosa* (16%), *Micrococcus sp*. (21%). as represented in fig 13. For Choba market, the percentage frequencies are as follows *Pseudomonas aueroginosa* (6%), *Salmonella* sp. (5%), *Proteus* sp., *Staphylococcus* sp. and *Escherichia coli* (13%), *Micrococcus* sp. (19%), *Bacillus sp* (26%). as shown in fig 14. From the ugba samples obtained from Rumuosi market, the percentage frequency of bacterial isolates includes *Pseudomonas aueroginosa*, *Staphylococcus* sp., *Escherichia coli* (7%), *Micrococcus sp*. (11%), *Staphylococcus aureus* and *Bacillus sp*. (22%). Lastly the percentage frequency for ugba samples from Alakahia markets recorded *Micrococcus* sp. (4%), *Proteus sp*. (5%), *Escherichia coli*, *Lactobacillus sp*, *Salmonella sp*. and *Streptococcus* sp. (9%), *Pseudomonas aueroginosa* (14%), *Staphylococcus aureus* (18%), *Bacillus sp* (23%).

The fungal isolates obtained from this study and their percentage frequencies include *Fusarium* sp. (9%), *Aspergillus flavus* (9%), *Penicillium* sp. (10%), *Aspergillus niger* (19%), *Candida* sp. (24%), *Saccharomyces cerevisae* (29%) as shown in Figure-17.

The presence of *Pseudomonas* sp. is of serious concern, as it known to be an opportunistic pathogen and it is linked to infections related to mortality among seriously ill and immune compromised individuals .

Bacillus sp. are found in the soil but have been linked to some clinical manifestation such as self-limiting food poisoning, ocular infections, endocarditis, bacteremia and meningitis and they have Enterotoxin production potential[17].

Escherichia coli are indicator organism,are indicative of faecal contamination and poor hygiene, there are different strain with potent virulence and toxic factors. They are linked to urinary gastrointestinal and urogenital ailments of humans[18].

Also the significant level of *Staphylococcus* sp. contaminated observed from this study are worth-noting because they were reportedly carried by 30-50% healthy humans as normal flora and one of the common implicated bacteria in hospital acquired infections [19] They are also linked to different human ailments ranging from minor skin infections such as boils, pimples to a number of life threatening diseases like bacteraemia and sepsis[20]. Staphylococcus has however, previously been reported to be involved in the fermentation of ugba (their number decreasing after 72 hours of fermentation) [1,7,21.] This may account for their presence in the present sample.

Aspergillus sp. which was the most frequently isolated fungi in the ugba samples is among the most abundant and widely distributed organisms on earth , member of this genus are saprophytic moulds reside in the environment without causing disease[9] *Aspergillus flavus* which was obtained in this study have also been isolated from peanuts, corns, grains and other foods[9].

Penicillium sp. is uncommon pathogens in humans and has also been reported as a common opportunistic pathogen causing systemic penicilliosis in AIDS patient in Thailand, Southern China and other part of Southern Asia[9]. It is therefore important to develop a strategy to antagonize their growth and survival in foods in order to neutralize the potential of these organisms serves as agents of food borne diseases.

Proximate analysis showed that ugba is highly proteinceous, which is an indicates that the food is naturally prone to high microbial load since microorganisms grow well in proteinaceous environment. The crude protein was observed to be 29.30 for nylon wrapped and 15.51% for leaf wrapped. In previous studies, ugba has been found to contain 36.2-43.89% crude protein which contains about 20 amino acids[22,23] This study recorded carbohydrate 3.45% for nylon wrapped and 16.08% leaf wrapped, this difference in the carbohydrate percentage could be due to the slight difference in the production and fermentation processes by different producers in different locations as carbohydrate decreases as fermentation increases.

The colour is a very important characteristic in assessing the overall quality of a product, these parameters reflect the overall quality in terms of the production process, raw materials used. The colour of the ugba samples ranged from brown to light brown and black in some wraps.

The aroma of all the ugba samples for the various markets where significantly similar which indicates that the wrapping materials does not impact an unpleasant flavor on the samples, any possible difference in the aroma might be as a results of deterioration of the ugba samples.

The pH of the ugba samples ranged from the highest 8.60 (RsN1) to the least 7.30 (RrN1), this is attributed to abundant production of ammonia during the fermentation due to protein hydrolysis and deaminase activity [15]and the temperature ranged

from the least 28.3ºC (AL2) to the highest 31.0ºC (RrL1) this variation can be attributed to the various storage conditions which can determine the type of microorganism present The proximate analysis of the ugba samples shows a slight difference between leaf and nylon wrapped ugba samples.

Conclusion

The study indicates that ugba wrapped with leaf had more microbial load than that wrapped with nylon and this could be due to microorganisms already present in the leaf being used to wrap the ugba and the degradation of the leaf by microorganisms. It is essential that one uses the right method of storage for ugba as the ubiquity of microorganisms can never be over emphasized; hence some packaging materials (nylon and leaf) promote their rapid growth.

Furthermore ,the microbial composition of ugba samples show reoccurrence of the same organisms and as such it could be said that organisms isolated are normal flora or resident microbes resulting from cultural, harvest practice, fermenting or unhygienic handling of ugba by sellers but rarely from preservation method or materials rather the method of preservation used would encourage proliferation of these microorganism or reduce their growth. The use sterile packaging materials and proper storage condition and duration enhances the overall safety of ugba. Standard evaluation should be done on locally processed food product and GMP (Good Manufacturing Practices) should be emphasized to reduce microbial contamination which could lead to food poisoning.

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