BACTERIOLOGY AND PROXIMATE COMPOSITION OF UGBA FROM MINNA MARKET AFTER PROLONGED FERMENTATION

[1*]MOHAMMED ABDULLAHI, [2]MUHAMMAD MUHAMMAD AND [3]SULAIMAN MOHAMMED

[1]Department of Microbiology, Faculty of Natural Sciences, IBB University Lapai, Niger state, Nigeria
[2]Department of Microbiology and Biotechnology, Faculty of Science, Federal University Dutse, PMB7156 Jigawa State, Nigeria
[3]Department of Biological Sciences, Faculty of Science Gombe State University, PMB127 Gombe, Nigeria
*Corresponding Author: ibrahimnusaiba2@gmail.com

ABSTRACT

Different fermentation time are used in production of varieties of Ugba. This affects bacteriological and nutritional quality of this popular and much preferred fermented food product in Nigeria. In this study, 10 Ugba samples collected from Minna market were further fermented for 2 days in the laboratory. Bacteriology and proximate composition of the laboratory fermented sample was evaluated and compared to unfermented original Ugba sample. Total bacterial count ranged from 63.00×10^4 CFU/g in laboratory fermented Ugba and 39.00×10^4 CFU/g in the Original unfermented samples. Proximate composition showed 53.61±0.01 % – 38.87±1.18 % Moisture, 2.26±0.14% – 2.4±0.22% Ash, 5.01±0.17% – 6.03±0.57 % Fat, 1.93±0.46% – 2.89±0.06 % Fibre, 27.37±1.18% – 23.15±1.31% Protein and 9.81±1.62% – 26.65±0.52% Carbohydrate in fermented and unfermented Ugba samples respectively. Bacterial load was higher in the fermented sample compared to original sample and *Bacillus* species identified the most occurring bacterial. Other less populated bacteria identified are *Escherichia coli, Pseudomonas*, *Streptococcus* and *Klebsiella* species. Proximate analysis revealed significant increase in moisture, protein contents and a decrease in carbohydrate with increased fermentation time. Prolonged fermentation increases the protein content of Ugba but enhances contamination and rapid spoilage.

Keywords: Fermentation time**,** *Bacillus*, Proximate composition, Microbial load

INTRODUCTION

Ugba, a well-known native food in the eastern communities of Nigeria is produced from seeds of African oil bean (*Pentaclethra macrophylla*) (Enujiugha and Akanbi, 2005). This local delicacy has gain wide acceptance and now consumed by other tribes in various Nigerian societies. Ugba have appreciated from subsistence home food to intermittent retail product and now exported to other African countries. Cultural openness and commercial interrelationship of the Igbo's increased the movement of this fermented food product to other parts of Nigeria and export abroad (Ogunshe *et al.,* 2012). The local delicacy is often called different names such as *Ukpaka, Apara* and *Ukana* by Igbo,

Yoruba, Efik and Ibibio, and the Benue people of North central states (Enujiugha and Akanbi, 2005). African oil been tree grew well in tropical climatic condition (Key, 1989). Thus, another reason for wide spread and consumption of Ugba in the South, mid-central and coastal areas of Nigeria.

Regardless of the method, preparation of this local food begins by softening the hard seed coats via boiling or roasted to ease removal (Sokari and Wachukwu, 1997; Okoro and Emefieh, 2018). Then, bare cotyledons is cooked before the final fermentation process. This further softens the cotyledons and dispose it to microbial transformation process. Furthermore, the cotyledons are washed few times in order to eliminate the bitter taste. Then, fermented for days to increase nutritional lushness and provide the desired taste (Falegan, 2014). This is accomplished by soaking the cotyledons in water for conversion via autochthonous microbial reactions. Reports from previous investigations describes the length of fermentation as a determinant for the nutritional richness of the Ugba product. Nonetheless, it also determined the type of Ugba meant to be produced. Njoku and Okemadu (1989) specified 3-5 days fermentation process to produce eatable Ugba. Whereas, Olasupo *et al.,* (2016) reported prolonging fermentation to about 6-10 days to produce Ugba meant to increase aroma of soups.

Unfamiliar culture and poor temperature control in the production of Ugba often increases certainty of product contamination. The rich amino acids and mineral components of Ugba permits proliferation of foreign microorganisms introduced via the utensil/water used in production (Nduko *et al.* 2017). Furthermore, *Bacillus* species was identified as the microflora involved in production of Ugba (Isu and Njoku, 1997; Eze *et al.,* 2014). However, investigations reported the presence of pathogenic enteric bacteria like *Escherichia coli*, *Pseudomonas, Streptococcus*, *Micrococcus Enterococcus* and *Proteus* species (Okorie and Olasupo, 2013; Ifeoluwa *et al.,* 2019). This reason emphasized the fact that microbial contamination cannot be totally ruled out in the production process.

Obviously, Ugba is nutritionally rich and contains all the essential nutrients and minerals required for healthy growth. This fermented food product is affordable to the teaming poor population in Nigeria. Ugba supplements the large quantity of the carbohydrate consumed by rural dwellers with protein and minerals. However, the presence of disease-causing microbiota accelerates pollution of the product by the foreign pathogenic microorganisms. Production of pollutants is facilitated by uncontrolled temperature condition that enhances enzyme activities in production process (Ifeoluwa *et al.,* 2019). Yet, prolonged fermentation used for production of specific type of the local food may affect its nutritional value or lead to spoilage. These health risks pose to the public through high consumption of this food is of serious concern. Therefore, the aim of this study is to evaluate the bacteria and examine the nutritional value of Ugba before/after extended fermentation period in the laboratory.

MATERIALS AND METHODS

Collection of the Samples

Ten (10) samples of the fresh Ugba were collected from different Igbo vendors selling the product in Minna market. The Ugba samples were randomly composed and transported in polythene bags to Microbiology Laboratory IBB University Lapai.

Sample Preparation

Preparation for experimental analysis begin as soon as the samples arrived the laboratory. Each sample in sliced pieces were separated into two equal halves. First halves of the samples labeled were returned to 4°C. The second halves were inserted into pre-labelled sterilized conical flasks and spontaneously fermented at 30°C for 2 days in an incubator (model and type).

Isolation of Bacteria from Ugba

Bacteriological analyses were carried out on the Ugba samples to assess the bacteria isolates. Two (2) grams of the fermented Ugba sample was aseptically dissolved in 20 ml distilled water (10% w/v) in a laminar flour (model number). The liquid sample was cultured at 30°C for 24 h and serially diluted by transferring 1 ml into 9 ml peptone water, and subsequently until 10^{-5} dilution was attained. One (1) ml of 10^{-5} dilution was spread-plated on nutrient agar (NA) plates and cultured at 30°C for 24 h. Then, detectible colonies were subcultured several times on fresh NA plates and enumerated as described by (Cheesbrough, 2006; Oyeleke & Istifanus, 2009).

Proximate Compositional Analysis

The methods described by Association of Official Analytical Chemists (AOAC) was used to determine proximate content of the samples before/after extension of fermentation process for 2 days. In these analyses, the moisture, ash, crude protein, crude fibre, crude fat and carbohydrate contents were evaluated (Helvich, 1990). Description of each methods follows

Moisture

Two (2) grams of the original sample and the one fermented for 2 days were weighed into separate pre-washed and weighed petri dishes. The weight of the samples + petri-dishes were affirmed. Then, the sample vaporised at 100°C in an oven to a constant weight and weighed again. The percentage difference in weight symbolised the moisture content of the samples. The weight difference was calculated by the formula:

$$\% \text{ Moisture} = \frac{\text{Weight (Petridish+Samp before Drying} - \text{Petridish+Sam after Drying)}}{\text{Weight of Sample}} \times 100$$

Ash

Two (2) grams of the sample was weighed into pre-washed and weighed crucibles. The weight of the sample + crucible was confirmed and the sample heated to ashes at 500°C in a muffle furnace for 3 hrs. Then, the sample was cooled in a desiccator and the difference in weight was determined and presented as the ash content. Percentage ash was calculated by the formula:

$$\% \text{ Ash content} = \frac{\text{Weight (Crucible+As} \quad - \text{Empty crucible)}}{\text{Weight (Crucible+Sample before Burning} - \text{Empty Crucible)}} \times 100$$

Crude Fibre

Two (2) grams of the sample was mixed in 200 ml solution of 2.50 g NaOH in a beaker and heated to boiling at 100°C. The filtrate was washed and dried to a constant weight at 100°C in an oven and weighed. Then, burnt to ashes at 500°C in a muffle furnace, cooled and reweighed. The weight loss was calculated in percentage and presented as the fibre content.

$$\% \text{ Crude fibre} = \frac{\text{Weight (Filtrate} - \text{Ash)}}{\text{Weight of original Sample}} \times 100$$

Crude Protein

Two (2) grams of the sample was introduced into 20 ml concentrated H_2SO_4 and 0.5 g Selenium and Hg_2SO_4 catalysts mixture in a Kjedahl flask. The mixture was digested via heating to a clear solution. This procedure was achieved by holding the flask into a digestion rack with retort stand and burner heating the sample solution under. The clear solution of the sample extract was made-up to 100 ml with distilled water after being allowed to cool. Then, 50 ml of the solution was made to go through distillation process to extract ammonia from the acid. Before distillation began, a receiving flask comprised of 5 ml 2% boric acid, 5 drops of Bromocresol blue and 1 drop of Methylene blue was positioned directly to the condenser of the Kjedahl distillation apparatus. A 5 ml of 40% NaOH solution was added. Drops of the distillate gradually collects into the receiving flask as the heating process continues. The distillate was finally titrated to pink colour with 0.01 N HCl acid solution. The nitrogen content was calculated using the formula:

% Nitrogen = Titre value × 0.01 ×14 × 4

Protein content was finally determined by multiplying Nitrogen value with a constant of 6.25 and expressed in percentage.

Crude fat

Two (2) grams of the sample was placed into a thimble of Soxlet extraction apparatus and carefully ploughed with a cotton wool. Then, 20 ml petroleum ether was introduced into the flask in the digestion rack and heated to boiling to extract the fat. The solvent was vaporised via heating at 100°C in a water bath and the fat in a conical flask was cooled in a desiccator. The fat was weighed and expressed as percentage fat content.

Carbohydrate

The percentage carbohydrate was determined by differential method 100 – (%Protein + % Moisture + % Ash + % Fat+ % Fibre)

Biochemical Tests

Morphology and biochemical characteristics were used to identify bacteria strains obtained from the samples. Gram stain reaction and biochemical tests like catalase, methyl red, Voges Proskauer (MR-VP), starch hydrolysis, coagulase, and indole as described by Ogbulie, *et al.,* (1998) and Cheesbrough, (2006).

Comparison of Microbial Load and Proximate Composition with Previous Study

Results obtained for prolonged fermentation in the laboratory were compared with previous work in which fermentation was conducted for 3 and 4 days. This was to check correlation/variance in findings as regard to the effect prolonged fermentation.

RESULTS

Bacterial Count in Ugba Sample

Figure 1 shows the disparity in mean CFU/g of the microorganisms found between the original sample and the one in which fermentation process was extended for 2 days in the laboratory. A total of 39.00×10^4 CFU/g of the bacterial was found in original Ugba. Whereas, the fermented sample had 63.00×10^4 CFU/g of the microorganisms. These results disclosed higher amount of the microbes in the sample in which fermentation was extended for 2 days compared to the unfermented samples. Biochemical tests revealed the presence of *Bacillus* species as the dominant bacteria in four samples (A_1, A_5, B_4 and B_5). Whereas, *Escherichia coli, Staphylococcus*, *Pseudomonas and Streptococcus* species were found more dominant in six samples of the product (A_2, A_3, A_4, B_1, B_2 and B_3) (Table 1).

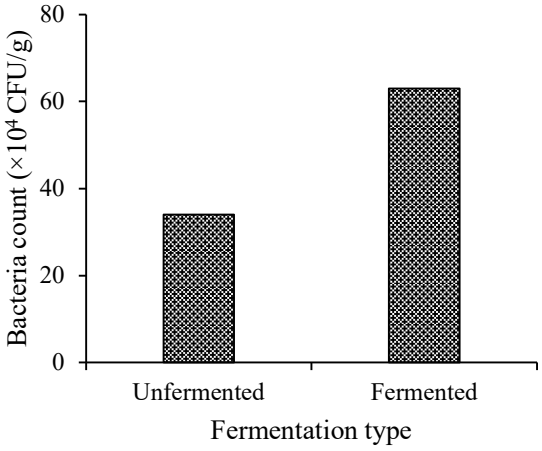

Figure 1: Comparison of the bacteria count in fermented and unfermented Ugba.

Table 1: Biochemical test for identification of the bacteria isolated from fermented and unfermented African oil bean seed (Ugba).

	Gram reaction	Shape	Catalase	Coagulase	Starch Utilization	Indole	Haemolysis on blood agar	Growth on MSA	MR	VP	Citrate	Probable bacteria
A_1	+	Rod	+	–	+	–	-	–	+	–	+	*Bacillus species*
A_2	+	Cocci	+	+	–	–	-	+	+	–	+	*Staphylococcus species*
A_3	–	Rod	+	–	–	+	-	–	–	+	–	*Escherichia coli*
A_4	+	Cocci chain	–	–	–	–	α	–	+	–	+	*Streptococcus species*
A_5	+	Rod	+	–	+	–	-	–	–	+	–	*Bacillus species*
B_1	–	Rod	+	–	–	–	-	–	+	–	+	*Pseudomonas species*
B_2	+	Cocci	+	+	–	–	-	+	+	–	–	*Staphylococcus species*
B_3	–	Rod	+	–	–	+	-	–	–	+	–	*Escherichia coli*
B_4	+	Rod	+	–	+	–	-	–	–	+	–	*Bacillus species*
B_5	+	Rod	+	–	+	–	-	–	+	–	+	*Bacillus species*

Key: +; positive results, –; Negative results

Prevalence of Bacteria in the African Oil Bean Seeds (Ugba)

Figure 2 shows the occurrence of the various bacterial strains in Ugba samples. *Bacillus* species had 40% occurrence, *Escherichia* sp and *Staphylococcus* sp 20%, and *Pseudomonas* and *Streptococcus* 10%. These results depict higher number of *Bacillus* species in the samples compared to other strains found in the sample.

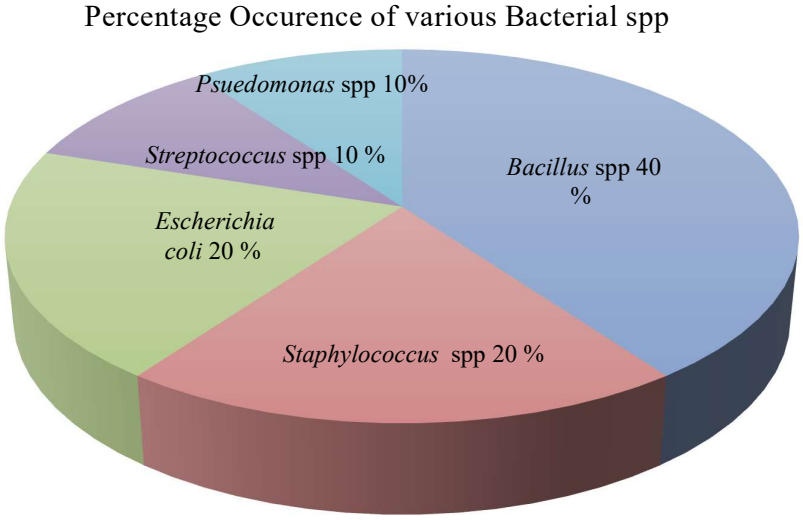

Figure 2: A pie chart illustrating the percentage occurrence of different bacterial Fermented Ugba.

Proximate Composition

Figure 3 shows the difference in nutritional content between the original Ugba sample and the one that was further fermented in the laboratory. Moisture content of 38.87±1.18 % and 53.61±0.01 % were detected in the original and the fermented Ugba samples respectively. The result showed a considerable high moisture in the fermented Ugba and indicates increasing moisture content with increased fermentation period. Ash content in both samples was low owing to 2.4±0.22% and 2.26±0.14% found in the original and laboratory fermented Ugba. Similar result was observed for the fat content in both samples. The original Ugba had 6.03±0.57 % and fermented Ugba showed 5.01±0.17% of the fat in the samples. Fat was higher in quantity in both samples compared to ash. However, a slight reduction in fat content was observed with increased fermentation period. Crude fibre was the lowest nutritional component found in both samples with 2.89±0.06 % and 1.93±0.46% detected in the original and fermented Ugba samples respectively. This indicates a slightly high reduction in the fibre content. For Crude protein, 23.15±1.31% was found in the original Ugba sample and 27.37±1.18% observed in the laboratory fermented sample. Thus, indicates increase in protein content with increased fermentation process. The carbohydrate content of the original Ugba sample (26.65±0.52%) was noticeably higher than that found in fermented sample (9.81±1.62%). This result indicates a

greater reduction of carbohydrate with extended fermentation period. Prolonged fermentation of the Ugba impacted the nutritional content by increasing moisture and protein contents. However, the influence mostly affected the carbohydrate content due to greater reduction in quantity. A slight/no variation in the ash, fat and fibre contents was also observed.

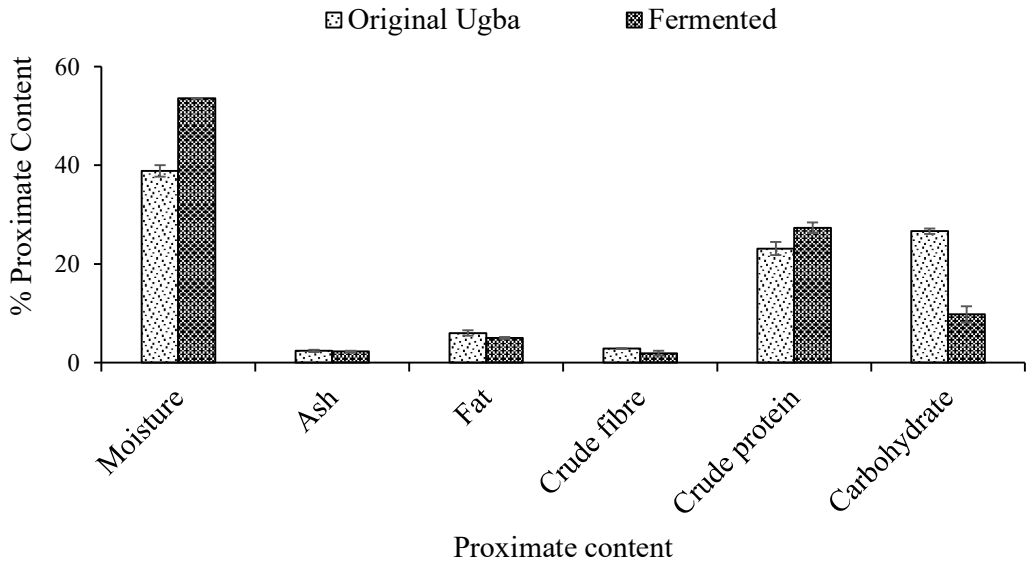

Figure 3: Proximate composition of original and the Ugba sample whose fermentation was extended in the laboratory.

Comparison of Microbial and Proximate Composition to Previous Study

Comparison of the proximate compositions demonstrates increase in moisture, protein and ash with a corresponding decrease in carbohydrate and fat content with increased fermentation time (Table 2). Whereas, microbial load irrespective of the fermentation time shows higher amount of *Bacillus* species compared to other bacterial found in all researches (Table 3). These observations indicate agreement in the findings and suggested that prolonged fermentation have great effect on the nutritional and microbial load of Ugba.

Table 2: Comparison of the proximate composition with previous works.

S/N	Fermentation days	Moisture %	Ash %	Carbohydrate %	Protein %	Fat %	Fibre %	Reference
1	3	49.77	1.09	7.87	13.17	10.51	17.64	Okorie & Olasupo, (2013)
2	3	34.34	6.12	15.03*	16.45	19.72	7.34	Eze *et al.,* (2014)
3	4	33.70*	8.83*	3.55	29.30*	18.32*	5.75*	Anyanwu *et al.,* (2016)
4	3	41.22	0.67	10.79	14.27	10.23	7.07	Ome & Olaoye, (2019)
5	5	53.61*	2.26*	9.81*	27.37*	5.01*	1.92*	This study

*; Increase in proximate composition with fermentation days

Table 3: Comparison of microbial load with previous works.

S/N	Isolates	Microbial Occurrence in Ugba Samples/Fermentation Days					
		Ogueke *et al.*, (2010) (g^{-1})	Nwamaka et al., (2010) (CFU/g^{-1})	Eze *et al.*, (2014) (%)	Anyanwu *et al.*, (2016) (%)	Obi, (2018) (%)	This study (%)
1	*Bacillus* species	9.2×10^8/3	17.7×10^7/4	17.9/3	95/4*	18.3/3	40/5*
2	*Streptococcus* species	Nil	Nil	12.5/3	Nil	10.4/3	10/5
3	*Staphylococcus* species	2.0×10^3/3	6.9×10^9/4	16.9/3	80/4*	13.0/3	20/5*
4	*Escherichia coli*	Nil	5.0×10^8/4	5.4/3	35/4*	4.30/3	20/5*
5	*Pseudomonas* species	Nil	Nil	4.5/3	Nil	4.30/3	10/5

*; Fermentation days with more increase in microbial load

DISCUSSION

The higher microorganisms discovered with extended fermentation of Ugba depicts increased microbial load. This indicates rapid multiplication of the microorganisms and increase in their number with prolonged fermentation period. This result was in agreement with that reported by Ogueke *et al.,* (2010). Perhaps, fast microbial growth was facilitated by the large amino acids, proteins and minerals released by metabolic degradation of the Ugba, which provides sufficient growth requirement. Similarly, the action of various enzymes successively degrades Ugba into aggregate of the nutrients. Thus, enriches the medium with sufficient nutrient that could be utilised by the microorganisms for rapid cell multiplication. Detection of *Bacillus* species in 40 % of the samples upholds previous report of *Bacillus* species being the dominant microflora responsible for conversion of the raw material into Ugba (Okorie and Oladapo, 2013).

However, discovery of enteric bacteria such as *Escherichia coli, Pseudomonas*, *Streptococcus* and *Klebsiella* species suggests contamination of the product. Contaminants might have been obtained from the water and utensils utilised in preparation of the product. Accordingly, Okorie *et al.,* (2017) reported entry of contaminants via unhygienic environmental condition, fermentation without knowledge of specific microorganisms involved as well as utensils used during production. Alternatively, departure of the product from manufacture centres to the market could be another route. Buyers often contacts the product shedding microorganisms during movement within the market (Anyanwu *et al.,* 2016).

Proximate analysis revealed great variation in the moisture, carbohydrate and protein content of the Ugba sold around the market with prolonged fermentation. The increase in moisture content of the fermented sample was about 15 % higher, signifying high moisture increase facilitated by further fermentation. This might be due to rapid microbial growth, which enhanced hydrolysis and transformation of the carbohydrate to liquid. According to Yang *et al.,* (2017), microbial and enzymatic hydrolysis of the carbohydrate loosens the

structural components in the carbohydrate transforming them into moisture. This was clearly seen in the semi-liquid product obtained. The findings agree with results reported by Ogueke *et al.,* (2010) and Sanni *et al.,* (2000). Furthermore, it is important to note that the presence of high moisture indicates possibility of the spoilage of the sample with further fermentation process. Because high moisture allows further contamination and favors microbial growth and activities (Sanni *et al.,* 2000). Thus, enhances synthesis of toxins by the inherent pathogenic species that may have gained access into the fermented food product.

Low ash content found was generally similar to the findings of Daniel *et al.,* (2018). However, a slight increase in the ash reported with further fermentation disagrees with a decrease in the ash content observed in this study. Okechuku *et al.,* (2012) reported lower ash content (0.24%) with further fermentation compared to 2.26% ash found in this study. A slight decrease in fat was observed with increased laboratory fermentation and this could be attributed to digestion of the fat. Lipase produced by the microbe's converts hydrophobic to hydrophilic groups by the influence of the temperature condition and enzymatic activities (Ilowefah *et al.,* 2015). Decrease in the crude fibre totally agrees with the findings of Igbabul *et al.,* (2014). This might be linked to microbial bioconversion of the carbohydrate into protein. The increase in protein content with extended fermentation of Ugba was comparable to the report of Gong *et al.,* (2020) after 24 hour increased fermentation of gelatinized potato flour. The increase in the crude protein may be due to increased microbial population and degradative reaction as the fermentation proceeds (Gupta *et al.,* 2007). This facilitates release of more protein by the activities of the microbes into the Ugba. High reduction of the carbohydrate might be linked to the increased breakdown and conversion of the carbohydrate to moisture (Yang *et al.,* (2017). From these results, a significant increase in moisture and crude protein, and reduction in carbohydrate was observed with extended fermentation of Ugba in the laboratory for 2 days. However, the changes in ash, fat and crude fibre with further fermentation was not large.

Protein increase is facilitated by microbial biosynthetic activities (Ome & Olaoye, 2019). Whereas, the moisture increase is triggered by microbial degradation which converts carbohydrate content to water via continuous hydrolysis (Obi & James, 2019). Therefore, these results establish the fact that prolonged fermentation improves the protein value of Ugba, which is an important substrate required for building new cells in a diet. However, the process enhances rapid spoilage of the fermented food product owing to favourable condition created for microbial growth.

Higher *Bacillus* species found in all studies compared to other bacteria is an indication of a microflora of oil bean seeds and ability to metabolise the raw material to Ugba (Eze *et al.,* 2014). However, other bacteria species detected in the 4^{th} and 5^{th} days of fermentation demonstrates proliferation of the pathogenic contaminants with increased fermentation period. Against the antimicrobials produced by *Bacillus* species (Nwagu *et al*., 2020), prolonged fermentation may have conferred resistance to these pathogenic species. Therefore, it is suggested that short fermentation period of about 3 -5 days should be sustained in production of Ugba because it reduces

further proliferation and production of contaminants. The use of fermentation up to ten days in production of certain kind of Ugba used in flavouring soup have great set back on the nutritional and microbial content of the fermented food product. Therefore, it should be discouraged. These findings also highlight the significance of *Bacillus* species in the production of Ugba. Thus, indicates the need for further investigation into the salient characteristics of *Bacillus* species that could be explored to enhance production of fermented food products.

CONCLUSION

The effect of prolonged fermentation time on bacteriological and nutritional quality of Ugba sold in Minna market was investigated in this study. The findings disclose high bacterial (63.00×10^4 CFU/g) in samples fermented in the laboratory compared to original unfermented sample (39.00×10^4 CFU/g) and *Bacillus* was the dominant strain in the sample. The presence of low concentration of *Escherichia coli, Pseudomonas*, *Streptococcus* and *Klebsiella* species was considered external contaminants obtained during production. Thus, prolonged fermentation time increased moisture and protein content of Ugba but facilitate rapid spoilage of the fermented food product. This demonstrates the need to reconsider fermentation time above 5 days used in production of certain variety of Ugba.

REFERENCES

Anyanwu, N. C. J., Okonkwo, O. L., Iheanacho, C. N., & Ajide, B. (2016). Microbiological and nutritional qualities of fermented ugba (Pentaclethra macrophylla, Bentham) sold in Mbaise, Imo State, Nigeria. *Annual Research & Review in Biology*, 1-8.

Cheesbrough, M. (2006). *District laboratory practice in tropical countries*. Cambridge university press. 146-157

Daniel, N. C., Chinedu, E. E., Ebere, E. C., & Emeka, A. C. (2018). Effect of Fermentation Time on the Proximate and Mineral Composition of Fermented African Oil Bean Seed 'Ugba'. *Sustainable Food Production*, 13.

Enujiugha V. N, Agbede J. O. (2000). Nutritional and anti-nutritional characteristics of African oil bean (Pentaclethra macrophylla benth). Seeds. *Application in Tropical Agriculture.* 5:11-15.

Enujiugha V. N, Akanbi C. T. (2005). Compositional changes in African oil bean (*Pentaclethra macrophylla* benth) seeds during thermal processing. *Pakistan Journal of Nutrition.* 4:27–31.

Eze, V. C., Onwuakor, C. E., and Ukeka, E. (2014). Proximate composition, biochemical and microbiological changes associated with fermenting African oil bean (*Pentaclethra macrophylla* Benth) seeds. *American Journal of Microbiology.* 2,674–681.

Falegan A. (2014). Enzymatic and haematological changes in rats (*Rattus norvegecus*) fed with defated ogiri (fermented *Citrullus vulgaris*) and melon seeds. *Journal of Natural Sciences Research.* 4 (17): 133-140.

Gong, S., Xie, F., Lan, X., Zhang, W., Gu, X., & Wang, Z. (2020). Effects of Fermentation on Compositions, Colour, and Functional Properties of Gelatinized Potato Flours. *Journal of Food Science*, *85*(1), 57-64.

Gupta, V., Sharma, A., & Nagar, R. (2007). Preparation, acceptability and nutritive value of rabadi-A fermented moth bean food. *Journal of Food Science and Technology-Mysore*, 44(6), 600-601.

Helvich, K. (1990). *Official Methods of Analysis* (No. 630.24 A88 1990). Association of official analytical chemists.

Igbabul, B. D., Amove, J., & Twadue, I. (2014). Effect of fermentation on the proximate composition, antinutritional factors and functional properties of cocoyam (*Colocasia esculenta*) flour. *African Journal of Food Science and Technology*, *5*(3), 67-74.

Ilowefah, M., Bakar, J., Ghazali, H. M., Mediani, A., & Muhammad, K. (2015). Physicochemical and functional properties of yeast fermented brown rice flour. *Journal of Food Science and Technology*, *52*(9), 5534-5545.

Keay, R.S. and Sawyer, R., (1995). Pearson's Composition and Analysis of Foods. Publ. Church Hill Livingstone, Edinburagh

Makanjuola, O. M., and Ajayi, A. (2012). Effect of natural fermentation on the nutritive value and mineral composition of African locust beans. *Pakistan Journal of Nutrition.* 11, 11–13.

Nduko, J. M., J. W. Matofari, Z. O. Nandi, and M. B. Sichangi. (2017). spontaneously fermented Kenyan milk products: A review of the current state and future perspectives. *African Journal of Food Science* 11 (1):1–11.

Nwagu, T. N., Ugwuodo, C. J., Onwosi, C. O., Inyima, O., Uchendu, O. C., & Akpuru, C. (2020). Evaluation of the probiotic attributes of Bacillus strains isolated from traditional fermented African locust bean seeds (Parkia biglobosa),"daddawa". *Annals of Microbiology*, *70*(1), 1-15.

Nwamaka, N. T., Chike, A., & Obiajulu, A. (2010). Role of bacteria isolates in the spoilage of fermented African Oil Bean Seed Ugba. *Pakistan Journal of Biological Sciences*, *13*(10), 497.

Obi Clifford Nkemnaso (2018). Effects of Thermal Processing and Fermentation on African Oilbean (Pentaclethra Macrophylla) Seeds during Ugba Production. *Bioscience and Bioengineering* 4 (2), 41-51

Obi, C. N., & James, M. U. (2019). Microbiological and Nutritional Changes Associated with the Thermal Processing and Fermentation of African Oilbean (Pentaclethra macrophylla) Seeds for ugba Production. *South Asian Journal of Research in Microbiology*, 1-15.

Ogbulie, J. N., Uwaezuoke, J. C., & Ogiehor, S. I. (1998). Introductory microbiology practical. *Nigeria: Springfield Publishers*, 24-157.

Ogueke, C. C., Nwosu, J. N., Owuamanam, C. I., & Iwouno, J. N. (2010). Ugba,

the fermented African Oilbean Seeds; its production, chemical composition, preservation, safety and health benefits. *Pakistan Journal of Biological Sciences*, 13(10), 489.

Ogunshe, A.A.O., Johnny, Z.M., Arinze, A.O., (2012). Effects of food spices on Gram-negative food indicator bacteria from some Nigerian ethnic fermented plant food condiments. *African Journal of Plant Science*. 6, 8–14.

Okechukwu, R. I., Ewelike, N., Ukaoma, A. A., Emejulu, A. A., and Azuwike, C. O. (2012). Changes in the nutrient composition of the African oil bean meal "ugba" (*Pentaclethra macrophylla* Benth) subjected to solid state natural fermentation. *Journal of Applied Bioscience* 51, 3591–3595.

Okorie, P. C., and Olasupo, N. A. (2013). Growth and extra cellular enzyme production by microorganisms isolated from *Ugba*- an indigenous Nigerian fermented food. *African Journal of Biotechnology.* 12, 4158–4167.

Okorie, P. C., Olasupo, N. A., Anike, F. N., Elemo, G. N., & Isikhuemhen, O. S. (2017). Incidence of enteric pathogens in ugba, a traditional fermented food from African oil bean seeds (Pentaclethra macrophylla). *International Journal of Food Contamination*, *4*(1), 12.

Okoro C. U and Emefieh C. V. (2018). Production of Ugba Cubes from Fermented *Pentaclethra macrophylla* Seeds. *Global Journal of Pure and Applied Sciences*, 24: 147-151

Olasupo, N. A., Okorie, C. P., & Oguntoyinbo, F. A. (2016). The biotechnology of Ugba, a Nigerian traditional fermented food condiment. *Frontiers in Microbiology*, *7*, 1153.

Ome, A. P., & Olaoye, O. A. (2019). A Study on Effect of Lactic Acid Bacteria Starter Culture on Physicochemical, Nutritional and Anti-nutritional Properties of Ugba, A Traditional Nigerian Fermented Food. *Journal of Medical and Applied Biosciences*, 11(2), 48 – 76.

Oyeleke, S. B., & Istifanus, N. (2009). The microbiological effects of hospital wastes on the environment. *African Journal of Biotechnology*, *8*(22): 6253-6257

Sanni, A. I., Ayernor, G. S., Sakyi-Dawson, E., & Sefa-Dedeh, S. (2000). Aerobic spore-forming bacteria and chemical composition of some Nigerian fermented soup condiments. *Plant Foods for Human Nutrition*, *55*(2), 111-118.

Sokari, T.G. and Wachukwu, C.K., (1997). Single rapid processing of African oil beans for Ugba production. *International Journal of food Sciences and Technology*, 32:77-79.

Victor N. Enujiugha (2003). Nutrient changes during the fermentation of African oil bean (*Pentaclethra macrophylla* Benth). *Pakistan Journal of Nutrition*, 2 (5), 320-323.

Yang, Q., Hui, X. G., Qiang, Y., & Hua, L. X. (2017). Improvement in corn flour applicability using lactic acid fermentation: A mechanistic study. *Starch-Stärke*, 69(5-6), 1600219