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A STUDY ON EFFECT OF LACTIC ACID BACTERIA STARTER CULTURE ON PHYSICOCHEMICAL, NUTRITIONAL AND ANTINUTRITIONAL PROPERTIES OF *UGBA*, A TRADITIONAL NIGERIAN FERMENTED FOOD

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ABSTRACT

The research was carried out with the aim of studying the effect of lactic acid bacteria on quality properties of *ugba* (fermented African oil bean seeds). The pure culture of lactic acid bacteria (*Lactobacillus plantarum*) isolated from *ugba* samples produced by traditional method, were successively screened and used (in single) to ferment African oil bean slices. The samples (*ugba* produced by spontaneous fermentation with that produced by LAB starter cultures) were subjected to physicochemical, proximate, anti nutritional and mineral analysis, and the mean values statistically analysed using a one-way Analysis of Variance at $P < 0.05$. The use of LAB starter culture in Sample B shortened the fermentation period from 72 h to 48 h with a significant reduction in pH (7.32 – 6.44) and increase in titratable acidity (0.068 – 0.097) as the fermentation time progressed, showing a favourable temperature (37°C) for the activities of lactic acid bacteria.There were significant differences ($P<0.05$) among all the samples in the pH and titratable acidity. The proximate composition of all the *ugba*samples had different levels of protein content (14.27%, 14.20%, 14.17%, 14.03%,

12.76%) respectively. Fat content (10.23% - 15.75%), moisture content (41.22% - 54.23%), ash content (0.67% - 2.89%), fibre content (7.07% - 9.03%) and carbohydrate content (10.79% - 25.73%), with Sample B (*ugba* produced by LAB starter culture) having the highest values in protein, fat and ash contents. There were significant differences ($P<0.05$) among all the samples in the proximate compositions. The anti nutritional factors of *ugba* produced by LAB starter culture had a significant reduction at the end of the fermentation process: tannins were reduced by >68%; oxalates were reduced by >90%; cyanide was reduced by >31% and phytic acid was reduced by >62% respectively. There were significant differences ($P<0.05$) among all the samples in the anti nutritional factors. The samples had different levels of mineral content: magnesium (162.05 – 189.06 mg/100g), calcium (270.10 – 298.19 mg/100g), sodium (11.35 – 20.65 mg/100g), potassium (16.80 – 24.73 mg/100g) and phosphorous (209.10 – 214.16 mg/100g), with Sample B (*ugba* produced by LAB starter culture) having the highest values in magnesium, calcium, potassium and phosphorous contents. There were significant differences ($P<0.05$) among all the samples in the mineral contents. It was concluded that pure cultures of *Lactobacillus plantarum* used as starter culture had good effect on the quality attributes of the fermented African oil bean slices.

Keywords: *Lactic acid bacteria, ugba, starter cultures, fermentation.*

INTRODUCTION

Ugbd refers to fermented African oil bean (*Pentaclethra macrophylla* Benth) seeds which are utilized by the Igbos and other ethnic groups in southern Nigeria as a delicacy and food

flavouring (Okorie and Olasupo, 2013b). It is a traditional food generally prepared in homes as a small family business and has significantly contributed to the diets of the people. Therefore, it serves as an important and cheap source of protein for people whose staple foods are deficient in proteins (Ogueke*et al.,* 2010). In other words, it constitutes an important nutritional contribution mainly as a source of protein, fats, and carbohydrate (Oboh and Ekperigin, 2004), and plays an economic, social and cultural role among the Igbos in the eastern part of Nigeria. Fermentation detoxifies the African oil bean seed with subsequent increase in nutrient availability and digestibility (Mbata and Orji, 2008).

Lactic acid bacteria (LAB) have a long history of safe use in fermented foods. Lactic acid bacteria (LAB) are industrially important organisms because of their fermentative ability as well as health benefits. However, they also have beneficial influence on the nutritional and sensory characteristics as well as on the standardization of end products (Olaoye and Onilude, 2009). Most lactic acid bacteria are considered as 'generally regarded as safe', GRAS for incorporation into food products (Silva *et al.,* 2002). The use of starter cultures has generally been recognized as one major way of ensuring product consistency and to a reasonable extent eliminates the problem of food-borne pathogens (Eman, 2009).

Unfortunately however, no lactic acid bacteria (LAB) starter cultures are commercially available yet for small scale processing of traditional African foods. The potential of starter cultures for fermentation on a household scale for most of our traditionally fermented foods has not yet been fully explored. The old tradition of using a portion of a

fermented product to start a new batch resembles the principle of starter culture in an empirical sense. Most commercial starter cultures originated from those food substrates to which they are applied today (Okorie and Olasupo, 2013a).However, much work is still necessary in this area to achieve remarkable success in improved product quality. Therefore, it was the aim of this study to determine quality properties of *ugba* samples produced by spontaneous fermentation and the use of LAB starter cultures.

MATERIALS AND METHODS

Collection of Samples

The African oil bean seeds (*Pentaclethra macrophylla* Benth) and the *Alchornealaxiflora*Benth leaves (*Akwukwo ugba* – the popular leaves for wrapping *ugba*) were purchased from different selling points at urban main market, Umuahia - Nigeria. The seeds were identified at the Department of Plant Health Management, Michael Okpara University of Agriculture, Umudike. The seeds were sorted, graded and washed in order to remove spoilt seeds, dust and extraneous materials from wholesome seeds prior to processing. Already produced *ugba* samples were purchased from the market while the control and LAB inoculated samples were prepared in the laboratory. The purchased *ugba* samples were collected in sterile polyethylene bags and sent immediately to the laboratory for analysis.

Lactic Acid Bacteria used

The LAB isolates used as starter cultures in this study are *Lactobacillus plantarum,* which have been isolated from *ugba* in a previous study (Olaoye *et al.*, 2018). The choice of the isolate from a number of LAB strains was based on their

ability to produce considerable quantities of lactic acid under a reduced pH and good fermentative activity exhibited.

Laboratory preparation of *Ugba* and the application of starter culture

The traditional and experimental procedures of Olaoye*et al*. (2017) of preparing *ugba* were employed in the laboratory to ferment the product. The processing of the large brown glossy seeds of the African oil bean to obtain '*ugbd* involves the following; 4 kg of raw African oil bean seeds were boiled in an autoclave at a temperature of 121°C and a pressure of 15 pounds per square inch (psi) for 1 h to soften the hard brown testa (shell). The heating was discontinued and the seeds were removed in batches and dehulled while hot. After dehulling, a local vegetable shredder (called *Nkwoo*in Ibo) - this was a perforated piece of metal which when the seed was ran over it at a certain angle, the seed came out in shreds) was used to shred the seeds. Then into boiling water in a pot, the shred of the African oil bean seeds were added and boiled with stirring at 5 min intervals, for 30 min. The boiled shred were poured into sterile sieve to drain out the hot liquor. Sprays of water were sprayed on the slices to completely remove hot liquor. Then the shreds were washed 3times, drained of wash water and steeped in distilled water in a pot and covered. The shreds were steeped for 10 h. After the steeping period, the shreds were vigorously stirred and poured into a sterile sieve (which has been autoclaved at temperature 121°C and pressure of 16psi) to completely drain out the steep water from the shreds. Ten millilitres (10ml) of the cultures containing (8.3 - 12.5 x 10^{10}Cfu/ml) was used to inoculate 100g sterile African oil bean shreds in singles and aseptically wrapped in sterile *Alchornealaxiflora* Benth leaves

(akwukwo ugba) washed and sterilized over a steam and lightly tied. The wrapped samples were kept in warm environment (34°C) to initiate fermentation and further left to ferment for 3 days (72 h) at room temperature (29 – 32°C) to yield '*ugba'.* The prepared sample (*ugba*) was labelled appropriately and reserved for further analysis.

Physicochemical Analysis

- **pH Determination**

A Digital pH meter (model Hanna 211) equipped with glass electrode was used for this purpose. It was used to measure the acidity and alkalinity of the *ugba* samples. It was first standardized using standard buffer solution of pH 4 and 7. The electrode was then rinsed with distilled water before immersing into the samples. Ten grams of the different samples were ground thoroughly in a mortar. This was then suspended in 100ml of distilled water to yield 10% solution. The pH of the suspension was measured using Digital pH meter (model Hanna 211).

- **Temperature Determination**

This was measured using calibrated mercury in glass thermometer (AOAC, 2002). A hole was bored through the wrapped samples and the bulb of the celcius thermometer was inserted through the hole and the temperature readings were taken directly from the thermometer.

- **Measurement of titratable acidity (%)**

The titratable acidity of the *ugba* samples was determined by the alkaline titrimetric method described by Eze *et al.* (2014). The titratable acidity expressed in percent (%) acid produced was determined by the titration of 10 ml of fermenting Ugba

sample dissolved in deionized water with 0.1N NaOH using phenolphthalein as an indicator until the end point (pink colour) is achieved. The percentage total titratable acidity (%TTA) was calculated as: 100/volume of sample x Normality NaOH used (0.0002) x Titre value

Proximate Analysis of the Samples

The parameters determined were moisture content, ash, crude protein, crude fat, crude fibre and carbohydrates. These parameters were determined according to methods of AOAC (2002).

Anti-nutrients Determination

The *ugba* samples were subjected to anti-nutritional analysis which include Hydrogen cyanide (HCN), tannin, phytate and oxalate using the methods of AOAC (2002) and Onwuka (2005).

Evaluation of Mineral Components

The mineral components of *ugba* samples evaluated were phosphorous, calcium, magnesium, sodium and potassium. These components were evaluated according to methods of James (1995) and AOAC (2002).

Statistical Analysis

The experimental design used was Completely Randomized Design (CRD). All data obtained were subjected to one-way Analysis of Variance (ANOVA) using Statistical Package for the Social Sciences (SPSS) version 22 for Windows; to determine any significant difference at 5% level (LSD) and reported as means of three replicates.

RESULTS AND DISCUSSION

The pH, temperature and titratable acidity of *ugba* samples fermented at different periods

The fermentation time, temperature as well as the hydrogen ion concentration (pH) and total titratable acidity (TTA) of the *ugba* samples are shown in Table 1. The samples were significantly different from each other at ($P<0.05$) in both hydrogen ion concentration (pH) and total titratable acidity (TTA). From the table, the pH of the *ugba* samples (Samples A and B) reduced from 7.32 to 6.44 with increase in total titratable acidity from 0.068 to 0.097 between 0 – 48 h of fermentation. There was also an increase in the temperature from 32 to 37°C. Sample D (market *ugba*-02) with salt fermented spontaneously had the lowest pH value (6.14) among the samples; while Sample C (market *ugba*-01) without salt fermented spontaneously had the highest value (7.69). The control Sample E produced in the laboratory had pH of (7.17) which was relatively the same with the value (7.69) for Sample C (market *ugba*-01). The pH and fermentation time (h) recorded in this work are in line with that reported by Enujiugha (2003), Okechukwu*et al.* (2012), Kabuo*et al.* (2013) and Okorie and Olasupo (2013b).

The total titratable acidity of *ugba* samples did not follow the same trend as Sample C (market *ugba*-01) without the addition of salt recorded the lowest value (0.041); while Sample B (*ugba* produced by LAB starter culture) had the highest value (0.097). The temperature of the fermenting samples (A-B) was found to increase slightly. This could be due to heat being generated as a result of exothermic reactions mediated by microbial enzymes. The temperature range recorded (32-37°C) favours the growth of lactic acid

bacteria. The proteolytic activities lead to an increase in ammonia nitrogen (Nwagu*et al.*, 2010) and with a resultant increase in pH (Mbajunwa*et al.*, 1998). The use of LAB starter culture in this work shortened fermentation period of *ugba* to 2 days (48 h) which is line with the period observed by Nwagu*et al.* (2011), Okorie and Olasupo (2013b), that the use of starter cultures reduced the fermentation of *ugba* from 3-4 days to 2 days (48 h) and starter cultures have been found to reduce fermentation time (Mbata and Orji, 2008) as well as improving product quality (Achi, 2005).

Table 1: The pH and titratable acidity of *ugba* samples

Samples	Fermentation Time (Hours)	Fermentation Temp. (°C)	pH	TTA (% lactic acid)
A	0	32	$7.32^{b} \pm 0.01$	$0.068^{c} \pm 0.002$
B	48	37	$6.44^{d} \pm 0.02$	$0.097^{a} \pm 0.001$
C	72	28	$7.69^{a} \pm 0.00$	$0.041^{e} \pm 0.001$
D	72	28	$6.14^{e} \pm 0.01$	$0.063^{d} \pm 0.003$
E	72	35	$7.17^{c} \pm 0.04$	$0.078^{b} \pm 0.002$
LSD	--	--	**0.12**	**0.004**

Results are Means of triplicate determinations with Total titratable acidity (TTA) expressed in (% lactic acid)

Values in the same column having the same superscripts are not significantly different (P>0.05)

Sample A = Unfermented African Oil bean Slices

Sample B = Fermented African Oil bean slices (*Ugba* inoculated with LAB starter culture)

Sample C = Market *Ugba*-01

Sample D = Market *Ugba*-02

Sample E = Control Sample

The Proximate Composition of *Ugba* Samp les

The result of the proximate composition of the *ugba* samples were shown in Table 2. The values of the proximate composition compared favourably with those found in literature though some were slightly lower or higher. The differences in the proximate composition may be due to dryness of the seed (whether freshly harvested - higher moisture level or dried and stored – lower moisture level), degree of maturity (higher fat and protein contents for matured seeds) whether raw, cooked and fermented or cooked and unfermented (Kabuo, *et al.*, 2013) that is, the condition of the seed before analysis. The fermentation of the sliced oil bean seed is known to produce nutritionally better products than the raw seeds (Enujiugha and Agbede, 2005; Enujiugha *et al.,* 2006). This work is in agreement with the finding as the proximate composition of the *ugba* samples followed the trend except for carbohydrate that had higher value than the fermented.

- **Crude protein**

The protein content of the *ugba* samples are shown in Table 2. The samples were significantly different from each other at ($P<0.05$) with Sample B (*ugba* inoculated with LAB starter culture) having the highest value (14.27%) and Sample A (unfermented African oil bean slices) having the least value (12.76%). The slight increases in crude protein value observed during fermentation could be due to the action of extra cellular enzymes produced by the fermenting micro-organisms or as a result of protein synthesis during the fermentation process. Lactic acid bacteria has been reported to improve the nutritional quality as well as reduce the toxicity of food materials (Drouault and Corthier, 2001). This could be the

reason for the increase in the protein content of the inoculated Sample B with the highest value (14.27%). Protein is important for growth in young ones, formation of enzymes, hormones, repair of worn out tissues, egg and milk production (Okechukwu*et al.,* 2012). The values obtained from this work are within the range reported by Okorie and Olasupo (2013b), who reported the protein content of 13.17 – 14.96%.

Table 2: Proximate composition of *ugba* samples

Samples	Crude Protein	Crude Fat	Moisture	Ash	Crude Fibre	Carbohydrate
A	$12.76^{e} \pm 0.02$	$12.55^{c} \pm 0.02$	$41.22^{e} \pm 0.02$	$0.67^{e} \pm 0.01$	$7.07^{e} \pm 0.02$	$25.73^{a} \pm 0.02$
B	$14.27^{a} \pm 0.01$	$15.75^{a} \pm 0.05$	$47.32^{d} \pm 0.02$	$2.89^{a} \pm 0.01$	$7.43^{c} \pm 0.02$	$12.34^{b} \pm 0.08$
C	$14.20^{b} \pm 0.02$	$10.23^{e} \pm 0.03$	$54.23^{a} \pm 0.03$	$1.51^{c} \pm 0.02$	$9.03^{a} \pm 0.04$	$10.79^{d} \pm 0.12$
D	$14.03^{d} \pm 0.03$	$14.53^{b} \pm 0.04$	$50.02^{c} \pm 0.03$	$2.50^{b} \pm 0.01$	$8.02^{b} \pm 0.02$	$10.90^{d} \pm 0.08$
E	$14.17^{c} \pm 0.01$	$12.46^{d} \pm 0.02$	$52.68^{b} \pm 0.02$	$1.36^{d} \pm 0.02$	$7.22^{d} \pm 0.02$	$12.11^{c} \pm 0.04$
LSD	**0.02**	**0.05**	**0.12**	**0.09**	**0.15**	**1.20**

Results are Means of triplicate determinations and expressed in Percentage (%)

Values in the same column having the same superscripts are not significantly different (P>0.05)

Sample A = Unfermented African Oil bean Slices

Sample B = Fermented African Oil bean slices (*Ugba* inoculated with LAB starter culture)

Sample C = Market *Ugba*-01

Sample D = Market *Ugba*-02

Sample E = Control Sample

- **Crude fat**

The fat content of the *ugba* samples are shown in Table 2. The samples were significantly different from each other at (P<0.05) with Sample B (*ugba* inoculated with LAB starter culture) having the highest value (15.75%) and Sample C (market *ugba*-01) without the addition of salt having the least value (10.23%) in Table 2. The increase in the fat content means more calories for man or animal using the fermented substrates as food/feed supplement. Cooking and fermentation give significant increase ($p < 0.05$) in crude fat content when compared with raw sample (Mbajunwa*et al.,* 1998). Lactic acid bacteria have been reported to improve the nutritional quality as well as reduce the toxicity of food materials (Drouault and Corthier, 2001). This could be the reason for the increase in the fat content of the inoculated Sample B with the highest value (15.75%). The observed values in this study are in concordance with the findings of Okorie and Olasupo (2013b), Kabuo*et al.* (2015), who reported the fat content of 10.51 – 15.50%, 6.5 – 12% respectively. Lipids helps in the production of energy (Okechukwu*et al.,* 2012).

- **Moisture content**

The moisture content of the *ugba* samples are shown in Table 2. The samples were significantly different from each other at (P<0.05) with Sample C (market *ugba*-01) without the addition of salt having the highest value (54.23%) and Sample A (unfermented African oilbean slices) having the least value (41.22%). The leaves used as wrappers that have pores which allow for the loss of water through evaporation and the metabolic activities of lactic acid bacteria in the fermenting *ugba* may have contributed to the low moisture observed in

Sample B (*ugba* inoculated with LAB starter culture). In addition, because a pile of leaves are used, it is believed that condensed water molecules are retained within the leaves and not on the fermenting mass. Addition of sodium chloride helps to reduce moisture content of food products (Ezeama, 2007) and this could be the reason Sample D (market *ugba*-02) with the addition of salt had a lower value than Samples C and E. Deterioration of *ugba* quality has been witnessed in form of sliminess, a common occurrence in spoilage of food with high residual moisture content(Mbajunwa*et al.*, 1998). The high moisture level has been suggested to predispose the product to rapid spoilage (Ogueke and Aririatu, 2004). Therefore, the shelf life of the product depends greatly on the level of water present in the food material. The findings recorded in this work are within the ranges reported by Okechukwu*et al.* (2012), Okorie and Olasupo (2013b), and Kabuo*et al.* (2015), who reported the moisture content of 28.61 – 51.88%, 42.01 – 49.77% and 44 – 52% respectively.

- **Ash content**

The ash content of the *ugba* samples are shown in Table 2. The samples were significantly different from each other at ($P<0.05$) with Sample B (*ugba* inoculated with LAB starter culture) having the highest value (2.89%) and Sample A (unfermented African oilbean slices) having the least value (0.67%) in Table 2. The increase of ash content by about 76% in the inoculated fermented product (Sample B) could be attributed to the increased metabolic activities of the fermenting microorganisms (*Lactobacillus plantarum*) and this could be the reason behind the tremendous yield in the mineral contents of the sample above other samples. The values recorded in this work are in concordance with the

findings of Enujiugha (2003), Enujiugha and Akanbi (2005), Chuku (2012), Balogun (2013), Okorie and Olasupo (2013b), Oyeleke*et al.* (2014), who reported the ash content of 2.1 – 2.9%, 1.11%, 2.3 – 2.6%, 1.08 – 1.33%, 0.98 – 1.68%, 1.94% respectively. For the increase observed in other samples, some of the biosynthetic mechanisms, especially those involving *Bacillus* species, are capable of synthesizing divalent metals (Enujiugha, 2003). The result of this finding agrees with that of Ogbo (2007) who reported a slight increase in ash contents of fermented African oil bean (*Pentaclethra macrophylla* Benth).

- **Crude fibre**

The fibre content of the *ugba* samples are shown in Table 2. The samples were significantly different from each other at ($P<0.05$) with Sample C (market *ugba*-01) without the addition of salt having the highest value (9.03%) and Sample A (unfermented African oilbean slices) having the least value (7.07%) in Table 2. Crude fibre content not being affected much by the fermentation could probably be due to the inability of the microbial agents to synthesize cellulases and hemicellulases for the hydrolysis of complex polysaccharides in the seeds (Enujiugha, 2003). The findings in this work are in agreement with the reports of Okereke and Onunkwo (2014), Eze *et al.* (2014), who reported the fibre content of 5.44 – 12.68%, 7.34% respectively.

- **Carbohydrates**

The carbohydrate content of the *ugba* samples are shown in Table 2. There were significant difference among samples A, B and E, at ($P<0.05$) and no significant difference between samples C and D ($P>0.05$). Sample A (unfermented African

oilbean slices) had the highest value (25.73%) and Sample C (market *ugba*-01) without salt had the least value (10.79%) in Table 2. The carbohydrate value of *ugba* has been reported to have decreased during cooking and fermentation (Mbajunwa*et al.,* 1998). The depletion of carbohydrate in the fermented *ugba* samples could be due to the active breakdown of the nutrient for carbon source by fermenting organisms during fermentation period. The observations in this study is also in agreement with the report made by Kabuo*et al.* (2013), that loss in carbohydrate may be as a result of leaching of soluble carbohydrates (sugars) and also the utilization of some of these sugars by fermenting microorganisms as carbon source for growth and metabolic activities. Carbohydrates helps in the production of energy (Okechukwu*et al.,* 2012). The results recorded in this work are in concordance with the findings of Enujiugha (2003), Enujiugha and Akanbi (2005), Chuku (2012), Eze *et al.* (2014), Okorie and Olasupo (2013b), Oyeleke*et al.* (2014), who reported the carbohydrate content of 16.6 – 19.8%, 17.48%, 10.3 – 15.5%, 15.03%, 7.82 – 9.66%, 10.30% respectively.

The anti-nutritional factors of *ugba* samples

The anti-nutrients present in the *ugba* samples are shown in Table 3. These anti-nutrients areconsidered important due to their toxicity. At the end of the fermentation process, phytic acid was reduced by >62%; oxalates were reduced by >90%; cyanide was reduced by >31% and tannins were reduced by >68% respectively.

- **Tannin content**

The tannin content of the *ugba* samples are shown in Table 3. The samples were significantly different from each other at

(P<0.05) with Sample A (unfermented African oilbean slices) having the highest value (0.744mg/100g) and Sample B (*ugba* produced by LAB starter culture) having the least value (0.234mg/100g) in Table 3. Boiling, washing and slicing as well as fermentation has been reported to reduce tremendously the tannin content of African oilbean seeds after (2 days) 48 h (Onwuliri*et al.,* 2004). Tannin in food depresses growth by decreasing protein quality and digestibility (Osagie*et al.,* 1996). Fortunately, the levels of tannin detected in these samples would not affect the nutritional potential of *ugba*. The findings in this study are within the ranges reported by Enujiugha and Akanbi (2005), who reported the tannin content of 0.22 – 0.38mg/100g but much lower than the values reported by Onwuliri*et al.* (2004) and Oyeleke*et al.* (2014), who reported the tannin content of 1.40 – 6.80mg/100g, 1.24mg/100g respectively.

Table 3: Anti-nutritional composition of *ugba* samples

Samples	Tannin (mg/100g)	Oxalate (mg/100g)	HCN (mg/kg)	Phytate (mg/100g)
A	$0.744^{a} \pm 0.003$	$2.63^{a} \pm 0.03$	$0.51^{a} \pm 0.01$	$0.16^{a} \pm 0.02$
B	$0.234^{e} \pm 0.001$	$0.24^{e} \pm 0.01$	$0.35^{b} \pm 0.02$	$0.06^{d} \pm 0.00$
C	$0.246^{c} \pm 0.001$	$0.98^{b} \pm 0.01$	$0.37^{b} \pm 0.03$	$0.12^{b} \pm 0.02$
D	$0.238^{d} \pm 0.002$	$0.88^{c} \pm 0.01$	$0.25^{c} \pm 0.03$	$0.08^{c} \pm 0.01$
E	$0.253^{b} \pm 0.002$	$0.27^{d} \pm 0.01$	$0.35^{b} \pm 0.02$	$0.07^{cd} \pm 0.01$
LSD	**0.008**	**0.02**	**0.02**	**0.01**

Results are Means of triplicate determinations and expressed in mg/100g except for Hydrogen Cyanide (HCN) in mg/kg

Values in the same column having the same superscripts are not significantly different (P>0.05)

Sample A = Unfermented African Oil bean Slices

Sample B = Fermented African Oil bean slices (*Ugba* inoculated with LAB starter culture)
Sample C = Market *Ugba*-01
Sample D = Market *Ugba*-02
Sample E = Control Sample

- **Oxalate content**

The oxalate content of the *ugba* samples are shown in Table 3. The samples were significantly different from each other at ($P<0.05$) with Sample A (unfermented African oilbean slices) having the highest value (2.63mg/100g) and Sample B (*ugba* produced by LAB starter culture) having the least value (0.24mg/100g) in Table 3. The values recorded in this work are below the tolerable limits. It has been reported that the lethal level in man is 2 – 5g and soluble oxalates has the ability to inhibit calcium, potassium and sodium – absorption probably due to their insolubility properties (Onwuka, 2005). The observation made in this work are in agreement with the findings of Enujiugha and Akanbi (2005), who reported the oxalate content of 0.81 – 2.79mg/100g.

- **Hydrogen cyanide (HCN)**

The cyanide content of the *ugba* samples are shown in Table 3. Samples A and D were significantly different from Samples B, C and E, at ($P<0.05$) and no significant difference between samples B, C and E ($P>0.05$). Sample A (unfermented African oilbean slices) had the highest value (0.51mg/kg) and Sample D (market *ugba*-02) containing salt had the least value (0.25mg/kg) in Table 3. The reduced values of the hydrocyanic acid noticed in the processing and fermentation in this work can be explained using the report of Onwuliri*et al.* (2004) which stated that food processing like soaking in water and cooking, drastically reduce the cyanide levels in

food. Onwuliri and Obu (2002), also noted that prolonged processing like soaking, cooking, discarding water used and removal of testa reduce cyanide too. This observation is in line with Amadi*et al.* (2011) who also noted that most toxicants are eliminated during processing and cooking. The values recorded in this work are much lower than the findings of Onwuliri*et al.* (2004), who reported cyanide content of 2.2 – 8.3mg/100g but higher than the values reported by Balogun (2013), who recorded a cyanide content of 0.02 – 0.04mg/100g respectively. However, the concentration of cyanide recorded in this work fell far below the tolerable limit of 3mg/100g asserted by Onwuliri*et al.* (2004).

- **Phytate content**

The phytate content of the *ugba* samples are shown in Table 3. Samples A and C were significantly different from Samples B, D and E, at ($P<0.05$) and no significant difference between samples B and E, D and E ($P>0.05$). Sample A (unfermented African oilbean slices) had the highest value (0.16mg/100g) and Sample B (*ugba* produced by LAB starter culture) had the least value (0.06mg/100g) in Table 3. This observation agrees with the fact that microflora enzymes which break down organic complexes to release anti nutrients which leach out into the surrounding medium are produced by fermentation or inoculation of starter cultures as was observed in this work. Lactic acid bacteria (LAB) has been reported to improve the nutritional quality as well as reduce the toxicity of food materials (Drouault and Corthier, 2001). The reduction in phytate level could be interpreted as one of the main reason behind the observed increases in the concentrations of these minerals in the fermented samples. The findings in this work are within the ranges reported by Balogun, (2013), who

reported the phytate content of 0.01 – 0.04mg/100g but much lower than the values reported by Enujiugha and Akanbi (2005), Oyeleke*et al.* (2014), who reported the phytate content of 1.16 – 2.11mg/100g, 0.72mg/100g respectively. Onwuliri *et al.* (2004), asserted that phytate levels can be reduced through soaking, dehydration and cooking.

The mineral elements composition of *ugba* samples

Results in Table 4 shows the mineral element composition of *ugba* samples which includes magnesium, calcium, sodium, potassium and phosphorus. From the Table, it could be observed that all the mineral contents were found to increase. Addition of LAB starter cultures to the oil bean seeds brought about a significant ($P<0.05$) increase in magnesium from 162.51mg/100g in the raw seeds to 189.06mg/100g in the inoculated sample (Sample B). However, the same process raised the concentrations of calcium, sodium, potassium and phosphorous in the inoculated sample. Animals need mineral elements for body functions such as bone formation, formation of eggshell, heart and muscle activities, nervous co-ordination, osmo-regulation and blood coagulation. This increase would ensure better mineral supply for the production of healthy animals (Okechukwu*et al.,* 2012) and necessary for teeth and bone development in children.

- **Magnesium**

The magnesium content of the *ugba* samples are shown in Table 4. Samples B, D and E were significantly different from Samples A and C, at ($P<0.05$) and no significant difference between samples A and C ($P>0.05$). Sample B (*ugba* produced by LAB starter culture) had the highest value (189.06mg/100g) and Sample C (market *ugba*-01) without the

addition of salt had the least value (162.05mg/100g) in Table 4. The high yield of magnesium observed in this study has great nutritional value. Magnesium maintains strong bones and tooth enamel, calms the nervous system, regulates heartbeat, strengthens digestion, maintains a healthy prostate in men, prevents swelling, keeps calcium soluble, prevents kidney stones and regulates the thyroid. It also improves urine retention and so helps to control incontinence in the elderly and prevent bed-wetting in children (Michael, 2009). The findings in this work are in agreement with the report of Balogun (2013), who reported the magnesium content of 126 – 180.20mg/100g but in close range with the values reported by Oyeleke *et al.* (2014), who reported the magnesium content of 210.70mg/100g respectively.

Table 4: Mineral composition of *ugba* samples

Samples	Magnesium	Calcium	Sodium	Potassium	Phosphorous
A	$162.51^{d} \pm 0.57$	$270.10^{e} \pm 0.09$	$11.35^{e} \pm 0.02$	$16.80^{e} \pm 0.03$	$210.24^{d} \pm 0.04$
B	$189.06^{a} \pm 0.06$	$298.19^{a} \pm 0.04$	$18.91^{b} \pm 0.02$	$24.73^{a} \pm 0.02$	$214.16^{a} \pm 0.02$
C	$162.05^{d} \pm 0.01$	$290.16^{c} \pm 0.02$	$16.21^{d} \pm 0.01$	$21.08^{c} \pm 0.02$	$210.41^{c} \pm 0.01$
D	$186.07^{b} \pm 0.06$	$296.10^{b} \pm 0.02$	$20.65^{a} \pm 0.04$	$23.42^{b} \pm 0.02$	$209.10^{e} \pm 0.04$
E	$174.64^{c} \pm 0.03$	$278.35^{d} \pm 0.01$	$17.71^{c} \pm 0.01$	$20.22^{d} \pm 0.02$	$211.65^{b} \pm 0.01$
LSD	**3.46**	**2.50**	**0.48**	**0.32**	**0.14**

Results are Means of triplicate determinations and expressed in mg/100g.

Values in the same column having the same superscripts are not significantly different (P>0.05)
Sample A = Unfermented African Oil bean Slices
Sample B = Fermented African Oil bean slices (*Ugba* inoculated with LAB starter culture)
Sample C = Market *Ugba*-01
Sample D = Market *Ugba*-02
Sample E = Control Sample

- **Calcium**

The calcium content of the *ugba* samples are shown in Table 4. The samples were significantly different from each other at (P<0.05) with Sample B (*ugba* produced by LAB starter culture) having the highest value (298.19mg/100g) and Sample A (unfermented African oilbean slices) having the least value (270.10mg/100g) in Table 4. Calcium content of 298.19mg/100g is high enough for normal body functions and is associated with desirable retention of body calcium to reduce osteoporosis, a condition of reduced bone density and the underlying case of bone fragility (Park *et al.,* 2005). It is also of paramount importance to the body as it normalizes nerve and muscle function, regulates heart-beat, enables blood clotting, helps to maintain a proper acid-alkaline balance, induces sleep and promotes skin health (Akinhami*et al.,* 2008; Michael, 2009). The values recorded in this work are higher than the findings of Enujiugha and Akanbi (2005), Balogun (2013), Oyeleke*et al.* (2014), who reported the calcium content of 208.92mg/kg, 26.12 – 28.20mg/100g, and 201.47mg/100g respectively.

- **Sodium**

The sodium content of the *ugba* samples are shown in Table 4. The samples were significantly different from each other at ($P<0.05$) with Sample D (market *ugba*-02) with the addition of salt having the highest value (20.65mg/100g) and Sample A (unfermented African oil bean slices) having the least value (11.35mg/100g) in Table 4. The higher sodium content recorded in Sample D (market *ugba*-02) could be attributed to the unknown concentration of NaCl added to the *ugba* by local processors of the product usually for taste improvement. Many fermented foods require the addition of salt to impart a salty taste as well as contribute to the flavour of the foods (Ezeama, 2007). Together with potassium, sodium regulates the sodium-potassium balance that affects water retention and stimulates kidney function (Michael, 2009). The values recorded in this work are within the range of values reported by Balogun (2013), who reported sodium content of 12.42 – 16.12mg/100g but higher than the findings of Enujiugha and Akanbi (2005), Oyeleke*et al.* (2014), who reported the sodium content of 172.06mg/kg, 2.80mg/100g respectively.

- **Potassium**

The potassium content of the *ugba* samples are shown in Table 4. The samples were significantly different from each other at ($P<0.05$) with Sample B (*ugba* produced by LAB starter culture) having the highest value (24.73mg/100g) and Sample A (unfermented African oilbean slices) having the least value (16.80mg/100g). The potassium values obtained in this study have a potential nutritional benefit. Together with sodium, potassium regulates the sodium-potassium balance that affects water retention and stimulates kidney function; it also promotes insulin secretion and is involved in nerve transmission and muscle contraction. In addition, potassium

promotes the disposal of the body's wastes, enhances mental alertness by increasing oxygen supply to the brain, and reduces blood pressure (Michael, 2009). The values recorded in this work are higher than the findings of Enujiugha and Akanbi (2005), who reported a value of 110.39mg/kg but lower than the values reported by Balogun (2013), Oyeleke*et al.* (2014), who reported the potassium content of 210.40 – 218.40mg/100g, 235.65mg/100g respectively.

- **Phosphorous**

The phosphorous content of the *ugba* samples are shown in Table 4. The samples were significantly different from each other at ($P<0.05$) with Sample B (*ugba* produced by LAB starter culture) having the highest value (214.16mg/100g) and Sample D (market *ugba*-02) containing salt having the least value (209.10mg/100g). Phosphorus is vital for the release of energy, converting glucose to glycogen (stored sugar) and helping to form lecithin. Phosphorus maintains strong bones and teeth, promotes growth and body repair, provides energy by helping to metabolize carbohydrates and fats, ensures proper functioning of nerves and maintains an acid-alkaline balance (Akinhami*et al.,* 2008; Michael, 2009). The values recorded in this work are in concordance with the findings of Balogun (2013), who reported the phosphorous content of 210.04 – 216.12mg/100g. However, the phosphorous content values recorded in this work were higher than the value reported by Enujiugha and Akanbi (2005), who reported a value of 291.02.39mg/kg but in close range (196.11mg/100g) with the findings of Oyeleke*et al.* (2014) respectively.

CONCLUSION

The use of LAB starter culture in this research work shortened fermentation period of *ugba* to 2 days (48 h). Moreover, there was an improvement in the proximate and mineral components. There was also a noticeable and significant reduction in antinutritional factors of the *ugba* sample inoculated with LAB starter culture showing that the study had a good effect on the quality of the fermented African oil bean slices.

REFERENCES

Achi, O. K. (2005). Traditional fermented protein condiments in Nigeria. *Africa Journal of Biotechnology.* 4 (13): 1612-1621.

Akinhami, T, F., Atasie, V. N. and Akintokun, P. O. (2008). Chemical Composition and Physiochemical Properties of Cashew Nut (*Anarcadiumoccidentale*) Oil and Cashew nut Shell Liquid. *Agricultural, Food and Environmental Sciences*. Vol. 2 Issue 2: 1-9

Amadi, B.A., Arukwe, U., Duru, M.K.C., Adindu, E.A., Ufornwa, E.C. and Odika, P.C. (2011). The Effect of Fermentation on Anti-nutrients, Carbohydrates and Vitamin Contents of *Pentaclethra macrophylla* Seed. *International Science Research Journal* 3: 74-77

Association of Official Analytical Chemists (AOAC). (2002), Official Methods of Analysis. The Association of Official Analytical Chemists International. 18th Ed. Arlington, VA.

Balogun, B.I. (2013). Evaluation of the Nutritional Potentials of Fermented Oil Beans Seed *Pentaclethra macrophylla*

Benth. *Production Agriculture and Technology Journal*, 9(2): 73-87.

Chuku, E.C. (2012). Effects of Wrapping Materials on Mould Growth and Proximate Composition of *Pentaclethra macrophylla* Benth. *Nigerian Journal of Mycology*, Vol. 5, 1-7

Drouault, S. and Corthier, G. (2001). Health effects of lactic acid bacteria ingested in fermented milk. *Vet Resources* 32(2): 101–117.

Eman, H.E.A. (2009). Starter culture development for improving safety and quality of Domiati cheese. *Journal of Food Microbiology,* 26:533–541

Enujiugha, V.N. (2003). Nutrient Changes during the Fermentation of African Oil Bean (*Pentaclethra macrophylla* Benth) Seeds. *Pakistan Journal of Nutrition,* 2 (5): 320-323

Enujiugha, V.N. and Agbede, J.O. (2005). Nutritional and anti-nutritional characteristics of African oil bean (*Pentaclethra macrophylla* Benth)seeds. *Journal of Applied Tropical Agriculture*; 5:11-14.

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

Enujiugha, V.N., Amadi, C, and Sanni, T. (2006). Amylase in raw and fermented African oilbean seed (*Pentaclethra macrophylla Benth*). *Journal of European Food Resources and Technology*, 214: 497-500.

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 Microbiological Research,* 2: No. 5: 138-142.

Ezeama, C. F. (2007). Food Microbiology; *Fundamentals and Applications.* Natural Prints Ltd, Lagos

James, C.S. (1995). *Analytical Chemistry of Foods*. Chapman and Hall, New York, USA, Pp. 41.

Kabuo, N.O., Uzuegbu, J.O., Ubbaonu, C. N. and Onyeka, E. U. (2013). The microorganisms and compounds influencing the organoleptic properties of *Ugba* (fermented *Pentaclethra macrophylla* Benth. seeds). *African Journal of Food Science* Vol. 7(2) pp. 25-34

Kabuo, N.O., Asoegwu, S.N., Nwosu, J.N., Onuegbu, N.C., Akajiaku, L.O. and Nwaimo, J.C. (2015). Assessment of Leaf-Type and Number of Leaves used in Wrapping on the Quality of "*Ugba*" (Fermented *Pentaclethra macrophylla* Benth Seed). *European Journal of Food Science and Technology,* Vol.3, No.1, pp.11-23

Mbajunwa, O.K., Akingbala, J.O., Mulongoy, K. and Oguntimein, G. (1998). Starter culture evaluation for the production of *ugba* from African oil bean seed (*Pentaclethra macrophylla*). *Journal of Science of Food and Agriculture*, 77: 127-132.

Mbata, T. and Orji, M.U. (2008). Process optimization in the production and preservation of *Ugba*, a Nigerian fermented food. *Int. International Journalof Microbiology,* 4: 2-6.

Michael, S. (2009). *NUTRIENTS A-Z*: A User's Guide to Foods, Herbs, Vitamins, Minerals &, Supplements. 5th ed. Carlton Books Limited, London.

Nwagu, T.N., Amadi, C. and Alaekwe, O. (2010). Role of Bacteria Isolates in the Spoilage of Fermented African Oil Bean Seed *Ugba*. *Pakistan Journal of Biological Sciences, 13: 497-503.*

Nwagu, T.N.T., Orji, M.U., Nwobodo, I. and Nwobodo, H.A. (2011). Mixed Microbial Flora as Starter Culture for Production of *Ugba* from African Oil Bean Seed. *Asian Journal of Biological Sciences, 4: 62-69.*

Oboh, G. and Ekperigin, M.M. (2004). Nutritional evaluation of some Nigerian wild seeds. *Journal of Nahrung Food*, 48: 85-87.

Ogbo, A.I. (2007). Fermented African oil bean (*Pentaclethra macrophylla* Benth) seeds, In: Clean Kitchen; Healthy Eating Issue No. 15, 2007. Pp 37 and 38.

Ogueke, C.C. and Aririatu, L.E. (2004). Microbial and organoleptic changes associated with *ugba* stored at ambient temperature. *Nigerian Food Journal*, 22: 133-140.

Ogueke, C.C., Nwosu, J.N., Owuamanam, C.I. and 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: 489-496.*

Okechukwu, R.I., Ewelike, N.C., Ukaoma, A.A., Emejulu, A.A. and Azuwike, C.O. (2012). Changes in the Nutrient Composition of the African Oil Bean Meal "*Ugbd*'

(*Pentaclethra macrophylla*) subjected to Solid State Natural Fermentation. *Journal of Applied Biosciences* 51: 3591-3595

Okereke, A.N. and Onunkwo, D.N. (2014). Effect of Processing Methods on Proximate Composition and Sensory Properties of "*Ugba*" (Fermented *Pentaclethra macrophylla* Benth), an African Oil Bean Product. *IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)*. Vol. 8, Issue 11, pp 32-38

Okorie, P.C. and Olasupo, N. A. (2013a). Growth and extracellular enzyme production by microorganisms isolated from *Ugba* - an indigenous Nigerian fermented condiment. *African Journal of Biotechnology:* Vol. 12(26), pp. 4158-4167

Okorie, P.C. and Olasupo, N. A. (2013b). Controlled fermentation and preservation of *UGBA* –an indigenous Nigerian fermented food. *SpringerPlus Open Journal* 2013 2:470.

Olaoye, O. A. and Onilude, A. A. (2009). Isolation and Biochemical Profiles of Numerous Strains of Lactic Acid Producing Bacteria from Various Parts of a Domestic West African Goat (*Capra hircus*). *Australian Journal of Basic and Applied Sciences*, 3(2): 460-466

Olaoye, O. A., Ome, A. P. and Lawrence, I.G. (2018). Biopreservative abilities of lactic acid bacteria isolated and characterized from a Nigerian traditional food *ugba. Journal of Recent Advances in Food Science*, 2018: 2(2): 85-96

Onwuka, G. I. (2005). *Food Analysis and Instrumentation*: Theory & Practice. Naphthali Prints, Lagos. pp. 133

Onwuliri, V.A. and Obu, J.A. (2002). Lipids and other constituents of *Vignaunguiculata*and *Phaseolus vulgaris* grown in Northern Nigeria. *Journal of FoodChemistry*, 78: 1-7

Onwuliri, V.A., Attah, I. and Nwankwo, J.O. (2004). Anti-nutritional factors, essential and non-essential fatty acids composition of *Ugba* (*Pentaclethra macrophylla*) seeds at different stages of processing and fermentation. *Journal of Biological Sciences*, 4: 671-675.

Osagie, A.U., Muzquiz, M., Borbano, C., Cuadrado, C., Ayet, G. and Castono, A. (1996). Anti-nutritional constituents of ten staple foods grown in Nigeria. *Journal of Topical Science*, 36: 109-115

Oyeleke, G.O., Odedeji, J.O., Ishola, A.D. and Afolabi, O. (2014). Phytochemical Screening and Nutritional Evaluation of African Oil Bean (*Pentaclethra macrophylla*) Seeds. *IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)*, Vol. 8, Issue 2, pp 14-17

Park, S., Kang, T., Kim, C., Han, J., Kim, S., Smith, R. H., Pike, L. M. and Hirshi, K. D. (2005). Genetic Manipulation for Enhancing Calcium Content in Potato Tuber. *Journal of Agricultural Chemistry,* 53: 5598-5603.

Silva, J., Carvalho, A.S., Teixeira, P. and Gibbs, P.A. (2002). Bacteriocin production by spray-dried lactic acid bacteria. *Letters in Applied Microbiology*. 34: 77–81

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