Journal homepage: www.fia.usv.ro/fiajournal
Journal of Faculty of Food Engineering,
Ştefan cel Mare University of Suceava, Romania
Volume XXI, Issue 1 - 2022, pag. 47 - 61

EFFECTS OF *Bacillus amyloliquefaciens* CULTURE CONCENTRATIONS ON MICROBIAL LOAD, PHYSICOCHEMICAL AND SENSORY PROPERTIES OF "IRU"-TYPE CONDIMENT FROM AFRICAN YAM BEAN (*Sphenostylis stenocarp*) SEEDS

***Pius Ifeanyi OKOLIE[1], Emilymary Chima OKOLIE[2], Sarah Abiola OGUNDIMU[1], Kudirat Aina AGBOOLA[1] and Maureen Chinwe OJINNAKA[3]**

[1] Federal University of Agriculture (FUNAAB), Abeokuta, Ogun State, Nigeria, okoliepi@funaab.edu.ng,
[2]National Biotechnology Development Agency of Nigeria, Lugbe, FCT-Abuja,
[3]Michael Okpara University of Agriculture (MOUAU), Umudike, Abia State, Nigeria,
*Corresponding author
Received 9th December 2021, accepted 20th March 2022

Abstract: *African yam bean seeds (Sphenostylis sternocarpa) Iru-like condiment was produced with 0.005 and 0.0075 g broth/g seed different concentrations of Bacillus amyloliquefaciens culture. The sample fermentation was conducted at 35 °C for 5 days. Proximate composition, water absorption capacity, oil absorption capacity, pH, and titratable acidity of the fermented samples were assessed using standard methods. Proximate composition of fermented African yam bean (AYB) condiment with 0.005 g broth/g seed inoculum concentration as determined were crude protein (6.86%-7.74%), crude fat (1.37%-1.71%), crude fiber (2.73%-4.81%), ash content (1.02%-1.59%), moisture content (9.71%-14.92%), dry matter (85.10%-90.28%), carbohydrate (70.43%-77.16%). The pH, total titratable acidity, water absorption capacity (WAC), and oil absorption capacity (OAC) of the fermented condiment were 6.76-7.60 ml, 0.07-0.09 ml, 0.50%-0.92%, and 0.55%-0.65% respectively. The fermented condiment produced with 0.0075 g broth/g seed inoculum concentration had its proximate composition as determined as crude protein (8.10%-8.53%), crude fat (1.81%-2.12%), crude fiber (2.83%-3.73%), ash content (1.23%-1.42%), moisture content (8.97%-12.81%), dry matter (87.19%-90.91%), carbohydrate (72.22%-76.13%) while its pH, total titratable acidity, WAC and OAC ranged as 7.10-8.76 ml, 0.06-0.11 ml, 0.38%-0.81% and 0.54%-0.84% respectively. The sensory acceptability scores reveal condiment samples from 0 hours as the best-preferred sample produced from the use of the inoculum concentrations. The study showed that AYB seeds condiments produced were significantly different in terms of different concentrations of starter culture used. Although the single starter culture did not deliver acceptable products during the fermentation, it played a few parts within the product quality.*

Keywords: *Inoculum concentration, Alkaline fermentation, Nutritional enhancement, Starter culture*

1. Introduction

Numerous underutilized leguminous seeds abound in the tropical regions of African countries and adequate characterization of these seeds has not been fully evaluated. Exploration for possible food product development from these underutilized food sources has been the focus of research interest aimed at alleviating food security problems in the African continent. In Nigeria, there has been an increasing shift in the utilization of these underutilized leguminous seeds to produce food condiments, a high protein-rich product. Protein from fermented leguminous plant seeds is an imperative wholesome nutrient source to people of most African nations [1]. Traditional fermented vegetable

DOI: https://doi.org/10.4316/fens.2022.006

proteins are routinely used as seasonings and condiments in African and Asian diets. These matured food varieties are attractive considering the benefits of further developed flavor, nutritive worth, and edibility over unfermented products [2, 3].

Nutrient bioavailability due to fermentation entails an increase in the essential amino acids and vitamins and a reduction in the availability of antinutrients. Some researchers have reported that fermented foods are associated with greater digestibility, increased flavors, and aromas as well as can impart some health-promoting significances [4, 5, 6]. Condiments also add to individual calorie and protein consumption and are frequently utilized as a modest meat substitute by low-pay families in the developing world [7].

African yam bean seed *(Sphenostylis sternocarpa)* is an underutilized plant species in most west African countries. African yam bean, also known as wild yam bean is an annual crop that belongs to the leguminous family and sub-family of *papilonacea sp.* It is a promising raw material for condiment production. Its nutritional composition is like that of most edible legumes such as soya bean, locust bean seed, and bambara nut seeds. It has been reported to contain about 21.10% protein, 5.70% crude fiber, 74.10% carbohydrate, 3.20% ash, 8.5% moisture, and 8.25% fat [8]. Several reviews have been done on the biochemistry and nutritional aspect of unfermented African yam bean seeds. Research studies on AYB seed have focused mainly on the utilization of the unfermented seeds as part of composite flour in confectioneries [9, 10, 11, 12, 13]. AYB seeds have limited utilization as food and food ingredients due to the presence of anti-nutrients such as tannin, phytic acid, saponin, and as well as its hard-to-cook phenomena. Despite the high nutritive value of the African yam bean seed, there is still a dearth of information on the utilization of fermented seeds for condiment production. The objective of this study is to evaluate the effect of the different concentrations of pure culture of *Bacillus amyloliquefaciens* on the physiochemical properties of "iru"-type condiments produced from African yam bean seeds under controlled fermentation.

2. Materials and methods

Source of raw materials

African yam bean seeds *(Sphenostylis stenocarpa)* used for this experiment were collected from the Isi-gate market in Umuahia, Abia state. The seeds were characterized as varieties TSS-5 and TSS-45 at IITA, Ibadan. The seeds were collected in polythene bags, aerated for 24 h, and stored in the refrigerator (6± 2 °C) until use.

Bacterial culture characterization and purification

Starter culture (*Bacillus amyloliquefacien*) was obtained from natural fermented AYB seeds. The organism was characterized genotypically using combined molecular techniques of PCR amplification of 16S-23S rDNA intergenic transcribed spacer (ITS-PCR) gene region together with repetitive sequence-based PCR (rep-PCR) and 16S rRNA gene sequencing to generate a unique molecular sequence for the isolate. The sequence was subjected to a blasting program on the NCBI site for isolate identity. The isolate was maintained on a Tryptone soy agar (TSA; Oxoid CM131, Basingstoke, UK) slant in the refrigerator until use.

Preparation of starter inoculum culture.

Starter culture preparation was conducted according to the modified method of [14]. Glycerol stored pure culture of *Bacillus amyloliquefacien* strain was plated on Tryptone Soy Agar (TSA; Oxoid CM131, Basingstoke, UK) plate and incubated overnight at 30 ± 2 °C. The *Bacillus* strain was subcultured in 50 ml of Tryptone Soy Broth (TSB; Oxoid CM129, Basingstoke, UK) in 100 ml conical flask and incubated at 30 ± 2 °C for 24 hours with an incubator shaker. The turbid cultures were centrifuged with a refrigerated centrifuge (Centurion, Scientific Ltd, UK) for 10mins at the speed of 10,000rpm. Cell pellets were harvested and re-suspended in 5 ml of 0.9% sterile normal saline (containing 9 g l^{-1} NaCl; pH 7.0). The number of viable cells in the resuspension was determined with a spectrophotometer (T60UV, PG Instruments Ltd, UK) at a wavelength of 540 nm and diluted it to 0.5 McFarland standard. The inoculum volume needed to inoculate 200 g of sterile AYB seeds to achieve the required concentration of 0.05 and 0.075 g broth/ g seed (containing 1.5x 108 cells per ml), was calculated.

Preparation of controlled fermented AYB seeds condiment

The modified method of [8] was used in the laboratory fermentation of AYB seeds under controlled conditions. Sorted African yam bean seeds were thoroughly washed and soaked in tap water for 6 h. The cotyledons were obtained by removing the seed coat by pressing the seeds between fingers. Approximately 100 g of the dehulled seeds were weighed and added into six 250 ml conical flasks. Sterilization of the seeds in the conical flask was done with an autoclave for 15 minutes at 121 °C. The sterile seeds were allowed to cool to room temperature and thereafter, inoculated with the bacterial culture. The inoculation was done carried out with different concentrations (0.005 g broth/g seed and 0.0075 g broth/g seed) of *Bacillus amyloliquefacien* containing 1.5 x 10^8 CFU/ml. The samples were fermented thereafter in a fermenter (incubator) at 30 ± 2 °C for 120 hours.

Analysis of the microbial growth during fermentation

Total viable bacteria during fermentation stages were enumerated by pour plate method with Tryptone soya agar (TSA, Oxoid CM0131, Basingstoke, Hampshire, UK). Samples (1g) of fermenting condiments from different inoculum concentrations were homogenized using 9ml of 0.9% of normal saline diluent in a stomacher bag. Replicate portions of ten-fold serial dilutions of samples were made for all samples collected for the two inoculum concentrations at different fermentation intervals (0h, 24h, 48h, 72h, 96h, and 120h). After serial dilution, 0.2 ml each of the serially diluted sample solution was pipetted into appropriately labeled Petri dish and autoclaved TSA media was poured aseptically into the different labeled Petri dishes. The plates were swirled gently in a planar circular motion to ensure uniform distribution and the media were allowed to solidify and incubated at 34°C for 24 hours. After incubation, colony growth was observed, and colony count was conducted.

Microbial count (CFU/g) = Colony count × Volume of Inoculum × Dilution factor × Correction factor.

Analysis of the physicochemical properties of fermented condiment

Fermenting samples were drawn at every 24 h intervals for enumeration of microbial load and determination of pH, titratable acidity, water absorption capacity, temperature, and oil absorption capacity.

Some portions of the sample were oven-dried at 60 °C for 2-3h, ground to powder using Kenwood blender, and stored in sealable nylon bags at 4° C for proximate composition analysis.

Proximate composition

Analysis of the proximate composition of the condiment samples for moisture, ash, total fat, crude fiber, and crude protein was carried out using the standard methods of the Association of Official Analytical Chemists [15]. Carbohydrate content was calculated by difference.

Titratable acidity

The total titratable acidity was assessed as described by [16]. Two (2) g of fermenting sample mash was homogenized with 20 ml with sterile distilled water. The sample mixture was filtered, and 10 ml of the filtrate containing 2 drops of 1% phenolphthalein solution as an indicator was titrated against 0.1 mmol l^{-1} NaOH solution at pH 8.1, and the mean titer value was taken. The titratable acidity of the sample was calculated as the percentage of lactic acid. One milliliter of 0.1 mmol l^{-1} NaOH solution was taken as equivalent to 0.009 g lactic acid.

pH

Ten (10) g of fermenting Otiru samples were taken aseptically and homogenized in 40 ml deionized water. Buffer calibrating solutions of pH 4 and pH 7 were used for initial calibration of the meter before sample readings were taken. (R1 – 02895 HANNA, Italy) [17].

Temperature

The temperature of the fermenting Otiru at the various period of evaluation was determined by inserting a sterile thermometer (sanitized by swabbing with 95 % ethanol) into each of the samples. The mercury-in-glass thermometer was used. Triplicate determinations were made in all cases [17].

Water/Oil absorption capacity

Water absorption capacity and fat absorption capacity of the fermenting samples were measured as recorded by [18]. Five grams of fermenting sample was ground to make slurry in 15 ml distilled water in determining the water absorption capacity (oil in the case of oil absorption capacity); the slurry was subjected to 20 min centrifugation at the speed of 4,000rpm. The free water (oil) after centrifugation was decanted, and the amount absorbed by the sample was calculated by difference.

Sensory evaluation

Sensory evaluation was conducted on the laboratory-produced condiment from African yam beans seed with different inoculum concentrations and a fresh market sample of "*iru"* (which served as a control sample). The sensory evaluation was done by 50 untrained panelists made up of male and female students selected from the Federal University of Agriculture, Abeokuta, Ogun State. The panelists were to access the appearance, color, texture, aroma, and overall acceptability of the fermented condiment using a 9-points Hedonic scale ranging from like very much (9) to dislike very much (1) as described by [19].

Statistical analysis

All analyses were performed in triplicate. Data were analyzed using SPSS (version 25) analysis of variance (ANOVA) and means separation was done with Duncan's multiple range test at $p \leq 0.05$.

3. Results and discussion

Bacteria isolation and identification

Presumptive *B. amyloliquefacien* was isolated during the natural fermentation of African yam bean seed condiment. The organism was examined to be Gram-positive rods, oxidase-negative, and catalase-positive. The isolate was also able to degrade citrate as their only carbon source. The isolate was able to hydrolyze starch and degrade casein and had a facultative mode of growth. Molecular characterization using combined techniques of ITS-PCR, rep-PCR, 16S rRNA gene sequencing, and sequence blasting, identified the isolate as *B. amyloliquefacien* DSM7 (T) with a 98.84 % sequence match and accession number of FN597644. Table 1 shows the result of the preliminary biochemical examination of the isolate.

Table 1:

Biochemical, Morphological and Molecular characterization of *Bacillus amyloliquefacien*

Characteristics/ biochemical Test	Observation
Gram Stain	Positive
Oxidase Test	Negative
Catalase Test	Positive
Starch hydrolysis	Positive
Casein degradation	Positive
Sugar fermentation:	
Glucose	Positive with gas
Lactose	Positive
Sucrose	Positive
Growth between 20 and 50 °C	Positive
Colony Morphology	Slimy, Big, Circular, Entire, Pulvinate, Dull, Rough, Opaque
Cell Morphology	Rods
Presumptive identity	*Bacillus amyloliquefacien*
Molecular identification (ITS-PCR/rep-PCR/16S rRNA gene PCR):	*B. amyloliquefacien* DSM7 (T) (FN597644) (98.84% Matched)

Microbial Analysis of controlled fermented AYB condiment from the first and second concentration of *Bacillus amyloliquefaceins*

Table 2 shows the result of the microbial load in the fermenting samples from the two inoculum concentrations. The result indicates that there was maximum growth of *Bacillus amyloliquefaceins* in the controlled fermented samples. Sample batch fermented with 0.005 g broth/g seed concentration of the inoculum recorded 2.1×10^9 cfu/g at 96 hours fermentation and 2.42×10^7 cfu/g at 72 hours. The microbial load increased as fermentation progressed from 24 hours to 48 hours but decreased at the 72 hour period. The highest cfu/g in the 0.0075 g broth/g seed concentration fermented sample batch was observed at 48 hours with 5.4×10^{10} cfu/g and the least microbial load was recorded at 24 hours with 3.5×10^8 cfu/g. The microbial load decreased slightly to 6.5×10^9 cfu/g as the fermentation progressed to 72 hours. It was observed that microbial growth increases with fermentation time. The rate of microbial development was observed to be comparatively higher in the AYB condiment samples fermented with 0.0075 g broth/g seed concentration of the inoculum starter. This higher fermentation rate could be attributed to the higher concentration of the starter. Table 2 shows that approximately the same volume of

microbial load obtained in 48 hours of fermentation of the AYB condiment batch fermented with 0.005 g broth/g seed concentration of the inoculum starter was recorded in sample batch fermented with 0.0075 g broth/g seed concentration of the inoculum starter at 24 hour of fermentation.

Table 2:

Results for Microbial Load Analysis

Fermentation time	0.005 g broth/g seed concentration	0.0075 g broth/g seed concentration
0 hour	ND	1.5×10^8
24 hours	3×10^7	3.5×10^8
48 hours	3.6×10^8	5.4×10^{10}
72 hours	2.42×10^7	6.5×10^9
96 hours	2.1×10^9	ND
120 hours	3.7×10^8	1.32×10^{10}

Values are mean of triplicate determination

Key**s:** CFU/g = colony forming unit per gram.

ND =Not determined

Effects of fermentation on Proximate composition of African yam bean seed condiment.

Table 3 shows the effect of fermentation with 0.005 g broth/g seed of inoculum concentration on the proximate composition of fermented AYB condiment. Condiment samples from 72 hours and 120 hours intervals had no significant difference in terms of their moisture content and dry matter. However, the mean values for moisture content and dry matter from other fermentation times were significantly different ($p \geq 0.05$). The percentage moisture content value of the sample was highest at 24 hours (14.92 %) but this decreased as fermentation progressed until 9.71% at 96 hours fermentation. The value for the moisture content increased finally to 10.95 % at 120 hours. The dry matter content increased significantly from 24 hours to 96 hours fermentation. The highest was 85.1% at 24 hours and 90.28% at 96 hours.

There was a reduction in fat content when compared to the raw sample. The values were significantly different ($p \geq 0.05$) except for the samples 48 hours and 96 hours fermentation intervals. The raw sample had 3.85% while the fat content between the unfermented and fermented AYB seeds ranged between 1.37% - 1.71%. This decrease in percentage fat content could be attributed to the utilization of fat by the microorganism [20]. There was a significant difference in crude ash content between raw seed and unfermented AYB seed as well as fermented AYB seeds. There was an increment when compared to the raw sample. The raw seed was 1.19% and the highest value obtained from the unfermented seed (UFS) was 1.59%. It was noticed that the value for the fermenting samples decreased as fermentation time increases. This is similar to the report of [21] during the production of a condiment from *Arachis hypogaea* Linn. This observation might be as a result of the de-hulling process of the beans since a higher percentage of the fiber and ash was concentrated on the seed hull which was removed during processing.

Crude fiber content was highest at 24 hours with 4.81% and lowest at 120 hours with 2.73%. The values were significantly different ($p \geq 0.05$). It was noticed that fiber content reduced as fermentation time increased, and this agrees with the result reported by [22] that fermented foods from legumes contain low fiber.

The protein content decreased when compared to raw samples. The highest protein value of the fermented sample was 7.74% at 96 hours and the lowest was 6.86% at 0 hours. The values were significantly different ($p \geq 0.05$). The observed reduction in value may be as a result of the breakdown of protein molecule structure in the seed leading to the release of ammonia which forms an excellent source of nitrogen for the growth of the fermenting organism [23, 20]. The carbohydrate content was highest at 120 hours with 77.16% and UFS with 70.43%. The values were significantly different ($p \geq 0.05$). There was an increase in the percentage carbohydrate content when compared to that of the raw sample. This difference could be attributed to the inability of the fermenting organism to degrade carbohydrates [20]. The result of the proximate composition of fermented condiment samples inoculated with 0.0075 g broth/g seed inoculum concentration shows that moisture content was highest at 96 hours with 12.81% and lowest at 8.97% at 24 hours (Table 4). The values were significantly different ($p \leq 0.05$). There was an increase in moisture content as fermentation time increases for 3 consecutive days (24 hours – 96 hours). This could be caused by the hydrolytic decomposition of the fermenting beans which had been earlier reported by [24]. There was a significant decrease in dry matter content from 24 hours to 96 hours of fermentation. The mean values for dry matter from the different fermentation times were significantly different ($p \leq 0.05$). The highest value was 91.00% at 24 hours and the lowest was 87.19% at 96 hours. There were significant differences in the fat content of samples fermented with 0.0075 g broth/g seed inoculum concentration across the different fermentation times. There was a reduction in fat content when compared to the value from the raw AYB seed sample. The raw sample had 3.85% while the highest of the fermented seed had 2.12%. This decrease in the percentage of fat content could be caused by the increased lipolytic enzyme activities by the microorganism. Fermenting organisms with high lipolytic enzymes potentials are good in degrading fat components into glycerol and fatty acid [23]. Crude ash content increased when compared to the raw AYB seeds. The raw seed was 1.19% and the highest value of the fermented seed was 1.42 at 24 hours. The values were significantly different ($p \geq 0.05$). It was noticed that the values decreased as fermentation time increases. This trend is similar to the report of [21] during the production of a condiment from locust beans. This observation might be a result of the de-hauling process of the beans since a higher percentage of the fiber and ash were concentrated on the seed hull which was removed during processing. Crude fiber content was highest at 120 hours with 3.73%. The values were significantly different among samples from different fermentation times. It was noticed that fiber content increased as fermentation time increased from 48 hours – 120 hours. The protein content decreased when compared to raw samples. There was no significant difference between fermented samples from 48 hours and 72 hours in their protein content. However, the highest protein content value of 8.68% (24 hours) was observed among the fermented samples while the unfermented

(UFS) recorded 8.1%. The observed reduction in value between the raw AYB seeds and the fermented samples could be due to the release of nitrogen from the free ammonia produced during the hydrolysis of protein components in the seed [23, 20]. The carbohydrate content was highest at 48 hours with 76.13% and lowest at 96 hours with 72.22%. The samples were significantly different. Ezekiel *et al.*, [20] had a similar result while working on fermented cotton seeds for Owoh condiment production. Observed changes in percentage carbohydrate content as the fermentation time changes aligned with the results of [25] on fermented cowpea and millet.

Table 3:

Results for Proximate analysis for 0.005 g broth/g seed concentration of the starter culture

Fermentation time	Moisture content (%)	Dry matter content (%)	Fat content (%)	Ash content (%)	Crude fiber (%)	Crude protein (%)	Carbohydrate (%)
UFS	$12.16^{d} \pm 0.01$	$87.88^{c} \pm 0.03$	$1.59^{c} \pm 0.04$	$1.59^{bc} \pm 0.03$	$3.84^{e} \pm 0.02$	$6.86^{a} \pm 0.02$	$74.52^{c} \pm 0.09$
24 hours	$14.92^{f} \pm 0.08$	$85.10^{a} \pm 0.07$	$1.45^{b} + 0.01$	$1.23^{d} \pm 0.01$	$4.81^{f} \pm 0.04$	$7.18^{c} \pm 0.05$	$70.43^{b} \pm 0.04$
48 hours	$10.92^{c} \pm 0.02$	$89.09^{d} \pm 0.03$	$1.69^{d} \pm 0.01$	$1.20^{d} \pm 0.02$	$3.46^{d} \pm 0.01$	$6.97^{b} \pm 0.02$	$75.83^{d} \pm 0.15$
72 hours	$10.57^{b} \pm 0.32$	$89.42^{e} \pm 0.32$	$1.37^{a} \pm 0.01$	$1.02^{a} \pm 0.04$	$2.90^{b} \pm 0.01$	$7.47^{d} \pm 0.01$	$76.66^{e} \pm 0.03$
96 hours	$9.71^{a} \pm 0.23$	$90.28^{f} \pm 0.02$	$1.71^{d} \pm 0.01$	$1.15^{c} \pm 0.01$	$2.89^{b} \pm 0.07$	$7.74^{e} \pm 0.02$	$76.75^{e} \pm 0.04$
120 hours	$10.59^{b} \pm 0.09$	$89.47^{e} \pm 0.09$	$1.61^{c} \pm 0.02$	$1.09^{b} \pm 0.02$	$2.73^{a} \pm 0.01$	$6.87^{a} \pm 0.02$	$77.16^{f} \pm 0.01$
Raw seed	$12.48^{e} \pm 0.06$	$87.54^{b} \pm 0.05$	$3.85^{c} \pm 0.00$	$1.19^{d} \pm 0.01$	$3.25^{c} \pm 0.02$	$14.59^{f} \pm 0.02$	$64.63^{a} \pm 0.01$

Values are the mean of triplicate determination. Mean values with different superscripts within the same column are significantly different ($P \leq 0.05$). ***UFS:*** *Unfermented African yam bean seed sample*

Table 4:

Result for Proximate analysis for 0.0075 g broth/g seed concentration of the starter culture.

Fermentation time	Moisture content (%)	Dry matter content (%)	Fat content (%)	Ash content (%)	Crude fiber (%)	Crude protein (%)	Carbohydrate (%)
UFS	$11.17^{e} \pm 0.07$	$88.86^{c} \pm 0.06$	$1.81^{a} \pm 0.01$	$1.24^{b} \pm 0.01$	$2.83^{a} \pm 0.03$	$8.10^{a} \pm 0.02$	$74.86^{d} \pm 0.01$
24 hours	$8.97^{a} \pm 0.06$	$91.00^{f} \pm 0.05$	$2.12^{e} \pm 0.05$	$1.42^{e} \pm 0.02$	$3.05^{c} \pm 0.03$	$8.68^{e} \pm 0.04$	$75.80^{e} \pm 0.21$
48 hours	$9.09^{b} \pm 0.03$	$90.91^{f} \pm 0.03$	$2.00^{d} \pm 0.02$	$1.37^{d} \pm 0.01$	$2.97^{b} \pm 0.02$	$8.45^{c} \pm 0.02$	$76.13^{f} \pm 0.04$
72 hours	$9.47^{c} \pm 0.08$	$90.57^{e} \pm 0.08$	$1.92^{c} \pm 0.01$	$1.35^{c} \pm 0.00$	$3.25^{d} \pm 0.04$	$8.42^{c} \pm 0.03$	$75.68^{e} \pm 0.07$
96 hours	$12.81^{g} \pm 0.02$	$87.19^{a} \pm 0.02$	$1.86^{b} \pm 0.02$	$1.23^{b} \pm 0.01$	$3.57^{e} \pm 0.01$	$8.29^{b} \pm 0.02$	$72.22^{b} \pm 0.01$
120 hours	$10.34^{d} \pm 0.06$	$89.68^{d} \pm 0.06$	$2.10^{e} \pm 0.01$	$1.34^{c} \pm 0.01$	$3.73^{f} \pm 0.01$	$8.53^{d} \pm 0.02$	$73.96^{c} \pm 0.01$
Raw seed	$12.48^{f} \pm 0.06$	$87.54^{b} \pm 0.05$	$3.85^{f} \pm 0.00$	$1.19^{a} \pm 0.01$	$3.25^{d} \pm 0.02$	$14.59^{f} \pm 0.02$	$64.63^{a} \pm 0.11$

Values are the mean of triplicate determination. Mean values with different superscripts within the same column are significantly different ($P \leq 0.05$). ***UFS:*** *Unfermented African yam bean seed sample*

Changes in the chemical composition of African yam bean seed condiment

Table 5 shows changes in the physiochemical composition of African yam bean seed fermented with 0.005 g broth/ g seed concentration of the starter culture. The fermenting time had a significant difference in the pH of the samples. The pH value ranged from 6.83% to 7.60% in the samples fermented with

0.005 g broth/g seed concentration of the starter. A rise in pH tending towards alkalinity was noticed in the fermented African yam bean seed as the fermentation time increases. This increase in pH can be explained as due to the proteolytic activity of *B. amyloliquefaciens* leading to the production of amines and free ammonia as a result of degradation of protein of the raw material. The produced ammonia gives the condiment its strong pungent and ammoniacal smell. The observed rise in pH agrees with the findings of some other researchers during fermentation of soybeans, Kariya seed, tamarind seed, and some vegetable proteins [26, 24, 20, 1, 16].

Table 5:

Changes in the physicochemical properties of fermented condiments with concentrations of *B. amyloliquefaciens.*

Fermentation Time	pH		WAC (ml)		OAC (ml)	
	BAFS1	BAFS2	BAFS1	BAFS2	BAFS1	BAFS2
UFS	$6.83^{a} \pm 0.05$	$7.10^{a} \pm 0.10$	$0.80^{d} \pm 0.00$	$0.56^{b} \pm 0.04$	$0.55^{a} \pm 0.01$	$0.80^{c} \pm 0.06$
24 hours	$6.76^{a} \pm 0.05$	$7.26^{b} \pm 0.05$	$0.50^{a} \pm 0.05$	$0.81^{c} \pm 0.09$	$0.57^{a} \pm 0.00$	$0.84^{c} \pm 0.04$
48 hours	$7.53^{c} \pm 0.05$	$7.46^{c} \pm 0.05$	$0.58^{ab} \pm 0.06$	$0.57^{b} \pm 0.02$	$0.65^{a} \pm 0.14$	$0.63^{ab} \pm 0.08$
72 hours	$7.36^{b} \pm 0.05$	$8.76^{d} \pm 0.05$	$0.92^{e} \pm 0.08$	$0.54^{b} \pm 0.04$	$0.57^{a} \pm 0.03$	$0.70^{b} \pm 0.04$
96 hours	7.23b ± 0.05	$8.23^{d} \pm 0.05$	$0.62^{bc} \pm 0.02$	$0.57^{b} \pm 0.09$	$0.57^{a} \pm 0.03$	$0.58^{ab} \pm 0.11$
120 hours	$7.60^{c} \pm 0.00$	$8.33^{e} \pm 0.05$	$0.70^{c} \pm 0.04$	$0.38^{a} \pm 0.09$	$0.65^{a} \pm 0.10$	$0.54^{a} \pm 0.00$

*Values are the mean of triplicate determination. Mean values with different superscripts within the same column are significantly different ($P \leq 0.05$). **BAFS1:** Condiment produced from fermented African yam bean seeds using a single starter culture of Bacillus amyloliquefacien inoculated at a concentration of 0.005 g broth/g seed; **BAFS2:** Condiment produced from fermented African yam bean seeds using a single starter culture of Bacillus amyloliquefacien inoculated at a concentration of 0.0075 g broth/g seed; **UFS:** Unfermented African yam bean seed sample. **WAC** = Water Absorption Capacity. **OAC** = Oil Absorption Capacity.*

Figure 1 reveals the result of the percentage changes in the titratable acidity of the AYB condiment. A gradual decline in the titratable acidity was noticed in the samples fermented with 0.005 g broth/g seed concentration of the starter. This decline in value was from 0.09% to 0.07% as the fermentation time increases. This drop-in titratable acidity could be attributed to the effect of a rising in pH value observed at the end of fermentation [20, 27]. Samples from 24h, 72h, 96h, and 120h fermentation times had no significant difference at $p \geq 0.05$ among them. These samples were significantly different from samples from 0h and 48h which were significantly the same. The titratable acidity results of samples from inoculation with 0.075 g broth/g seed concentration of the starter were significantly different concerning fermentation time. The titratable acidity decreases as the fermentation time increases and ranges from 0.11% to 0.06%. The titratable acidity from this concentration had an inverse relationship with the pH. The pH of the samples increases towards alkalinity with increasing fermentation time. The pH ranged from 7.10% to 8.33%.

The temperature of the fermenting samples was not significantly different for samples inoculated with 0.005 g broth/g seed concentration of the starter. The starter culture concentrations had no significant effect on the temperature of the samples. The mean temperature value ranged from 31.33 °C to 37.8 °C and 30 °C to 39 °C for batches of samples with 0.005 and 0.0075g broth/g seed concentrations respectively. The temperatures increase as the fermentation time increases irrespective of the inoculum concentration. There was a significant difference in the water absorption capacity of the AYB seeds fermented with 0.005g broth/g seed concentration. The WAC was

lowest at 24 hours (0.50 ml) and highest at 72 hours (0.92 ml) This indicates that WAC increases with fermentation time. However, for the samples fermented with 0.0075g broth/g seed concentration, there was no significant difference between the unfermented seeds and the fermented samples from 48 hours, 72 hours, and 96 hours fermentation times concerning their water absorption capacity. The WAC was lowest at 120 hours (0.38 ml) and highest at 24 hours (0.81ml).

There was no significant difference in the oil absorption capacity among all the AYB seeds samples fermented with for 0.005g broth/g seed concentration The OAC for this concentration had the lowest at 24 hours (0.57 ml) and highest at 48 hours and 120 hours (0.65 ml) while for 0.0075g broth/g seed concentration, the OAC was lowest at 120 hours (0.54 ml) and highest at 24 hours (0.84 ml). This shows that OAC increases with fermentation time for the first concentration and vice versa for the second concentration.

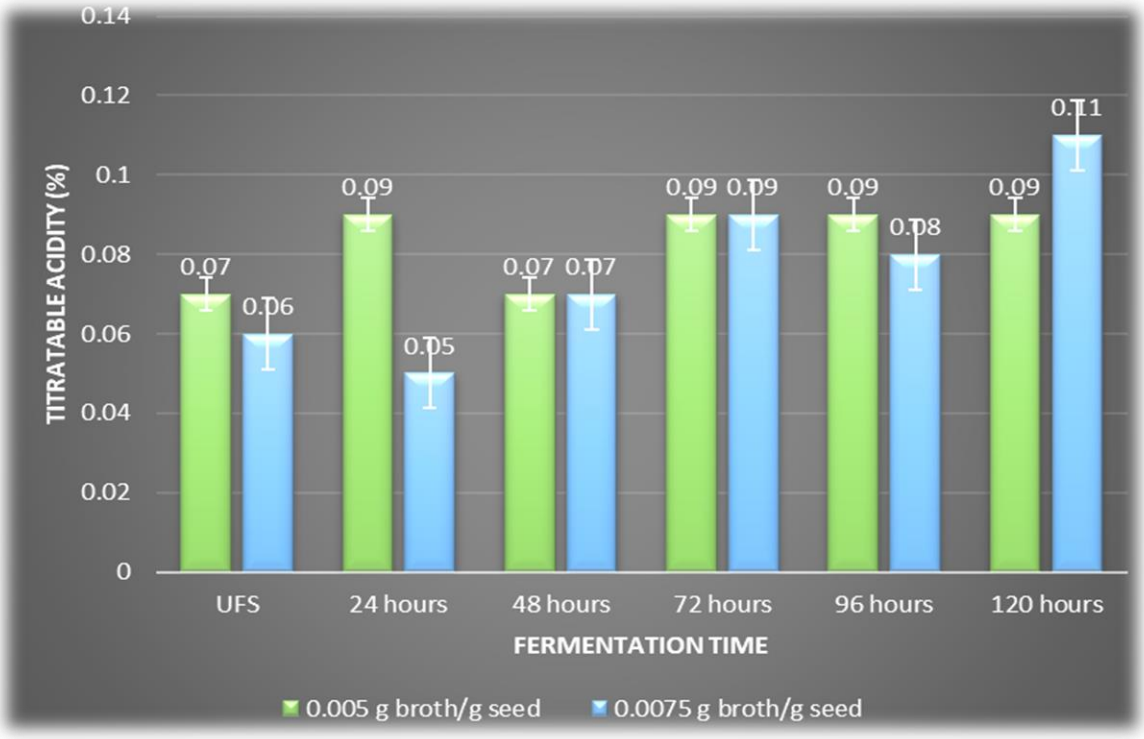

Figure 1: Change in percentage of lactic acid of AYB condiment samples fermented with 0.005 and 0.0075 g broth/ g seed concentrations of *B. amyloliquefaciens*

Effects of fermentation on Mineral composition of controlled fermented African yam bean condiment

The result of the mineral composition of the fermented condiment produced with different inoculum concentrations of the starter culture was presented in table 6. The sodium content of the controlled fermented condiment was between 2.16 - 2.87 mg/100g and 3.16 - 4.04 mg/100g for inoculum concentrations of 0.005 and 0.0075 g broth/g seed respectively. There was a reduction in the sodium content value of the fermented condiment when

compared to the raw AYB seed sample with 4.87 mg/100g. The mean values for the sodium content of the condiment across the fermentation time were significantly different ($P \leq 0.05$). The mean values for the magnesium content from the different inoculum concentrations were significantly different ($p \leq 0.05$). The magnesium content ranged from 152.98 - 160.79 mg/100g for samples fermented with inoculum concentrations of 0.005 g broth/g seed and 181.45 - 216.77 mg/100g for samples fermented with inoculum concentrations of 0.0075 g broth/g seed. There was an increase in value when compared to the raw sample (158.79 mg/100g). The phosphorus content of the fermented condiment was significantly different ($p \leq 0.05$) for both inoculum concentrations. The values for the phosphorus content were between 216.79 – 281.45 (mg/100g) and 291.50 – 324.50 (mg/100g) for the samples fermented with 0.005 g broth/g seed and 0.0075 g broth/g seed concentration of *Bacillus amyloliquefaciens* starter respectively. There was an increase in phosphorus content when compared to the raw sample with 277.43 (mg/100g). The increment in the phosphorus content from this study agrees with the research findings of [28, 29] on the production of Dawadawa condiments from Bambara groundnut and assessment of volatile flavor compounds from "Ugba" fermentation respectively.

The mineral contents determined were significantly affected with regards to the fermentation time and inoculum concentration.

Table 6:

Results for the Mineral composition of controlled fermented AYB condiment

time	Sodium (mg/100g)		Magnesium (mg/100g)		Phosphorus (mg/100g)	
	BAFS1	**BAFS2**	**BAFS1**	**BAFS2**	**BAFS1**	**BAFS2**
UFS	$2.87^{f} \pm 0.02$	$3.67^{b} \pm 0.28$	$157.07^{c} \pm 1.48$	$188.72^{d} \pm 0.03$	$281.45^{g} \pm .00$	$296.54^{d} \pm 0.03$
24 hours	$2.16^{a} \pm 0.00$	$4.04^{f} \pm 0.04$	$152.98^{a} \pm 0.21$	$206.11^{e} \pm 0.00$	$242.11^{b} \pm .00$	$301.65^{e} \pm 0.02$
48 hours	$2.67^{d} \pm 0.00$	$3.78^{c} \pm 0.00$	$161.09^{e} \pm 0.01$	$186.45^{c} \pm 0.00$	$273.12^{e} \pm .00$	$324.50^{g} \pm 0.00$
72 hours	$2.48^{b} \pm 0.00$	$3.16^{a} \pm 0.00$	$156.82^{c} \pm 0.03$	$216.77^{g} \pm 0.01$	$216.79^{a} \pm .01$	$287.67^{b} \pm 0.01$
96 hours	$2.61^{c} \pm 0.00$	$3.83^{d} \pm 0.01$	$154.15^{b} \pm 0.00$	$181.45^{b} \pm 0.00$	$261.31^{d} \pm .01$	$291.50^{c} \pm 0.01$
120 hours	$2.81^{e} \pm 0.00$	$3.89^{e} \pm 0.00$	$160.79^{e} \pm 0.02$	$212.67^{f} \pm 0.02$	$258.30^{c} \pm .00$	$312.53^{f} \pm 0.02$
Raw seed	$4.87^{g} \pm 0.01$	$4.87^{g} \pm 0.01$	$158.79^{d} \pm 0.00$	$158.79^{a} \pm 0.00$	$277.43^{f} \pm .01$	$277.43^{a} \pm 0.01$

Values are the mean of triplicate determination. Mean values with different superscripts within the same column are significantly different ($P \leq 0.05$).

BAFS1: *Condiment produced from fermented African yam bean seeds using a single starter culture of Bacillus amyloliquefacien inoculated at a concentration of 0.005 g broth/g seed;* ***BAFS2:*** *Condiment produced from fermented African yam bean seeds using a single starter culture of Bacillus amyloliquefacien inoculated at a concentration of 0.0075 g broth/g seed;* ***UFS:*** *Unfermented African yam bean seed sample*

Effects of fermentation on Anti-nutritional factors in controlled fermented AYB condiment

Table 7 shows the result of the antinutritional factors of the fermented AYB seeds condiment produced with different inoculum concentrations of the starter culture. There was a significant difference in the phytate content as fermentation time increases. The phytate content ranged between 2.20% - 2.88% and 0.34% - 3.46% for inoculum concentrations of 0.005 and 0.0075 g broth/g seed respectively. The result indicates a significant ($p \leq 0.05$) reduction in the concentrations of antinutrients

examined as the fermentation increases. The observation is in line with the trend of findings by [5] on the assessment of the antinutrient contents present in *Hura crepitans* seed. However, the mean values for phytate content observed in this study were higher when compared to the report of [30] on some varieties of velvet beans (*Mucuna spp*).

Tannin content was between 4.58% and 5.13% for 1st concentration and it was between 4.81% and 5.68% for 2nd concentration. These values are higher than the result of 0.80% reported by [31] for *Mucuna pruriens.* The values were significantly different (p> 0.05).

Trypsin inhibitor content was between 0.13% - 0.17% and 0.24% - 0.38% for 0.005 and 0.0075 g broth/g seed respectively. The mean values for the trypsin inhibitor content of the fermented AYB seeds from the two inoculum concentrations were significantly different ($p \leq 0.05$) concerning the fermentation time. There was a general reduction in the anti-nutritional content when compared to the raw sample.

Table 7:

Result for Anti-nutritional components of controlled fermented AYB condiment

Fermentation time	Phytate (%)		Tannins (%)		Trypsin inhibitor (%)	
	BAFS1	BAFS2	BAFS1	BAFS2	BAFS1	BAFS2
UFS	2.61^{d} ±0.00	0.34^{c} ± 0.01	5.13^{c}±0.02	5.46^{d} ± 0.01	0.15^{d} ± 0.00	0.31^{d} ± 0.00
24 hours	2.20^{a} ±0.01	3.19^{a} ± 0.03	4.80^{b}±0.05	4.81^{a} ± 0.00	0.13^{a} ± 0.00	0.28^{b} ± 0.00
48 hours	2.83^{e} ±0.01	3.66^{e} ± 0.01	4.62^{a}±0.01	5.36^{c} ± 0.03	0.14^{c} ± 0.00	0.24^{a} ± 0.00
72 hours	2.26^{c} ±0.01	3.46^{d} ± 0.01	4.76^{b}±0.10	5.64^{e} ± 0.02	0.17^{f} ± 0.00	0.30^{c} ± 0.00
96 hours	2.22^{b} ±0.00	3.27^{b} ± 0.06	4.88^{b}±0.10	5.19^{b} ± 0.01	0.13^{b} ± 0.00	0.34^{e} ± 0.00
120 hours	2.88^{f} ±0.00	3.28^{b} ± 0.00	4.58^{a}±0.06	5.68^{f} ± 0.00	0.16^{e} ± 0.00	0.38^{f} ± 0.00
(Raw seed)	4.87^{g} ±0.01	4.87^{f} ± 0.01	5.90^{d}±0.01	5.90^{g} ± 0.01	0.40^{g} ± 0.00	0.40^{g} ± 0.00

Values are the mean of triplicate determination. Mean values with different superscripts within the same column are significantly different ($P \leq 0.05$).

BAFS1: *Condiment produced from fermented African yam bean seeds using a single starter culture of Bacillus amyloliquefacien inoculated at a concentration of 0.005 g broth/g seed;* ***BAFS2:*** *Condiment produced from fermented African yam bean seeds using a single starter culture of Bacillus amyloliquefacien inoculated at a concentration of 0.0075 g broth/g seed;* ***UFS:*** *Unfermented African yam bean seed sample*

Sensory properties of fermented condiments

Table 8 shows the sensory score for the controlled fermented AYB condiment from different concentrations of *Bacillus amyloliquefaceins.* It was observed that for the 0.005 g broth/g seed inoculum concentration fermented samples, the 0-hour sample had the most preferred appearance with values of 7.62 while the least preferred was the 96 hours fermented sample with values of 5.96. For the sample batch fermented with 0.0075 g broth/g seed inoculum concentration, the 0 hours and control samples had the highest value with 7.62 and the lowest was 6.06 respectively. The highest recorded value for color was 7.28 from the 0-hour sample and the least was 6.32 at 48 and 96-hour fermentation for the samples fermented with 0.005 g broth/g seed inoculum concentration and the least was 6.32 at 72-hour fermentation for the batch fermented with 0.0075 g broth/g seed inoculum concentration. The 24-hour sample had the highest value for texture, 7.14 at 24-hour fermentation and the least was 1.83 at 96 hours for the 0.005 g broth/g seed inoculum concentration fermented samples while for the sample batch fermented with 0.0075 g broth/g seed inoculum concentration, the 24-hour

sample was mostly preferred with values 6.82 and the least preferred had values 6.18 at 120-hour fermentation. The samples with the highest values for aroma from the 0.005 g broth/g seed inoculum concentration fermented batch were samples from 0 and 24-hour fermentation, both values were 6.78 and the least was 6.10 at 120 hours fermentation while for the batch fermented with 0.0075 g broth/g seed inoculum concentration, the highest value was also 6.78 from the 0-hour sample and the least was 6.28 from 24-hour fermentation. The samples that were generally accepted for both concentrations were samples from 0 and 24 hours of controlled fermentation. The values were 7.32 and 7.22 for the batch fermented with 0.005 g broth/g seed inoculum concentration and 7.32 and 7.04 for the samples fermented with 0.0075 g broth/g seed inoculum concentration. This showed that even at the different levels of concentration of *B. amyloliquefacein* in the samples, 0 and 24 hours were more preferred in terms of appearance, color, texture, and aroma.

Table 8:

Result of the sensory score for controlled fermented AYB condiment from 1st and 2nd concentration

Fermentation Time	Appearance		Colour		Texture		Aroma		Overall acceptability	
	1st conc	2nd conc	1st conc	2nd conc	1st conc	2nd conc	1st conc	2nd conc	1st conc	2nd conc
0 hour	$7.62^{c}\pm1.24$	$7.62^{bc}\pm1.24$	$7.28^{c}\pm1.27$	$7.28^{c}\pm1.27$	$6.66^{bcd}\pm1.18$	$6.66^{ab}\pm1.18$	$6.78^{bc}\pm1.79$	$6.78^{ab}\pm1.79$	$7.32^{cd}\pm0.95$	$7.32^{b}\pm0.95$
24 hours	$7.20^{bc}\pm1.44$	$7.04^{bc}\pm1.17$	$7.12^{bc}\pm1.73$	$7.04^{bc}\pm1.16$	$7.14^{cd}\pm1.40$	$6.82^{ab}\pm1.25$	$6.78^{bc}\pm1.40$	$6.28^{a}\pm1.77$	$7.22^{bcd}\pm1.21$	$7.04^{ab}\pm1.27$
48 hours	$6.56^{ab}\pm1.74$	$6.82^{b}\pm1.46$	$6.32^{a}\pm1.84$	$6.80^{abc}\pm1.41$	$6.34^{ab}\pm1.67$	$6.52^{a}\pm1.48$	$6.18^{ab}\pm1.85$	$6.56^{ab}\pm1.51$	$6.80^{bcd}\pm1.48$	$6.68^{ab}\pm1.44$
72 hours	$6.38^{a}\pm1.78$	$6.46^{ab}\pm1.41$	$6.54^{ab}\pm1.64$	$6.32^{a}\pm1.58$	$6.46^{abc}\pm1.83$	$6.54^{a}\pm1.63$	$6.18^{ab}\pm1.94$	$6.36^{a}\pm1.69$	$6.60^{ab}\pm1.77$	$6.44^{a}\pm1.72$
96 hours	$5.96^{a}\pm1.81$	$6.64^{ab}\pm1.54$	$6.32^{a}\pm1.77$	$6.64^{abc}\pm1.54$	$1.83^{a}\pm5.80$	$6.76^{ab}\pm1.53$	$5.84^{a}\pm2.17$	$6.44^{ab}\pm1.88$	$6.00^{a}\pm2.02$	$6.78^{ab}\pm1.60$
120 hours	$6.54^{ab}\pm1.77$	$6.06^{ab}\pm1.99$	$6.48^{ab}\pm1.48$	$6.56^{ab}\pm1.75$	$2.14^{bc}\pm6.58$	$6.18^{a}\pm1.63$	$6.10^{ab}\pm2.01$	$6.34^{a}\pm2.06$	$6.66^{abc}\pm1.82$	$6.52^{a}\pm1.91$
Control	$7.60^{c}\pm1.52$	$7.62^{c}\pm1.51$	$6.92^{abc}\pm1.88$	$6.86^{abc}\pm1.89$	$1.65^{d}\pm7.30$	$7.24^{b}\pm1.47$	$7.22^{c}\pm1.92$	$7.16^{b}\pm1.91$	$7.40^{d}\pm1.79$	$7.34^{b}\pm1.79$

Values are mean of triplicate determination
Mean values with different superscripts within the same column are significantly different ($P \leq 0.05$)

4. Conclusion

Fermented condiments form an important nutrient-enhancing component of the diet in most African countries. The main effect of the fermentation time and inoculum concentration as well as their interaction effects on the physicochemical and proximate properties of the fermented African yam bean seed condiment shows that the physicochemical properties of fermented condiment were affected by inoculum concentration and fermentation time. The proximate composition of fermented AYB was lower than the raw seed which may be as a result of the low concentration of microorganisms added to the samples.

The inoculum concentration had a significant effect on the mineral and antinutrient factors of the fermented condiment. The mineral content was increased, and anti-nutritional factors were reduced.

5. Acknowledgments

The authors acknowledge the support of the management of Biotechnology Centre, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria in the provision of laboratory equipment for this research work.

Conflict of Interest:

The authors declare no conflict of interest

6. References

[1]. FAWALE, O.S., GBADAMOSI, S.O., IGE, M.M. & KADIRI, O. Effects of cooking and fermentation on the chemical composition, functional, and antinutritional properties of Kariya (*Hildergardia barteri*) seeds. *Wiley Food Science and Nutrition*, 5(6): 1106-1115, (2017).

[2]. QIN P., WANG T. & LUO. Y. A review on plant-based proteins from soybean: Health benefits and soy product development, *Journal of Agriculture and Food Research*, ***7*:** 100265, (2022), https://doi.org/10.1016/j.jafr.2021.100265.

[3]. PARKOUDA, C., NIELSEN D.S., AZOKPOTA P., OUOBA L.I.I., AMOA-AWUA W.K., THORSEN L., HOUNHOUIGAN J.D., JENSEN J.S., TANO-DEBRAH K. & DIAWARA B. The microbiology of alkaline fermentation of indigenous seeds used as food condiments in Africa and Asia. *Critical Reviews in Microbiology* **35(2):**139–56, (2009).

[4]. ADEWUMI, G.A., Health-Promoting Fermented Foods. In: Melton, L., Shahidi, F., & Varelis, P. (Eds.), *Encyclopedia of Food Chemistry*, **3:** 399–418. Elsevier. ISBN: 9780128140260 (2019).

[5]. MELINI, F.; MELINI, V.; LUZIATELLI, F.; FICCA, A.G. & RUZZI, M. Health-Promoting Components in Fermented Foods: An Up-to-Date Systematic Review. *Nutrients*, **11:** 1189. (2019). **h**ttps://doi.org/10.3390/nu11051189

[6]. ADEBO, O.A. African Sorghum-Based Fermented Foods: Past, Current and Future Prospects. *Nutrients* **12**: 1111. (2020), https://doi.org/10.3390/nu12041111

[7]. AHAOTU, I., ANYOGU A., NJOKU O.H., ODU N.N., SUTHERLAND J.P., & OUOBA L.I.I. Molecular identification and safety of Bacillus species involved in the fermentation of African oil beans (Pentaclethra macrophylla Benth) for production of Ugba. *International Journal of Food Microbiology***. 162 (1)**: 95–104, (2013).

[8]. JEFF-AGBOOLA, Y.A. Microorganisms Associated with Natural fermentation of African Yam Bean (*Sphenostylis sternocarpa* Harms) seeds for the production of Condiment. *Research Journal of Microbiology*. **2(11):** 816-823, (2007).

[9]. ADEBO, J.A.; NJOBEH, P.B.; GBASHI, S.; OYEDEJI, A.B.; OGUNDELE, O.M.; OYEYINKA, S.A. & ADEBO, O.A. Fermentation of Cereals and Legumes: Impact on Nutritional Constituents and Nutrient Bioavailability. *Fermentation*, ***8:*** 63. (2022). https://doi.org/10.3390/fermentation8020063

[10]. IDOWU, A. Chemical Composition and Sensory and Pasting Properties of Blends of Maize-African Yam Bean Seed. *Journal of Nutritional Health and Food Science*. 3: 1-6, (2015).

[11]. INYANG, U.E. & EYO, E.A. Effect of Soaking African Yam Bean Seeds in Unripe Plantain Peel Ash Solutions on the Nutrients, Anti-nutrients, and Functional Properties of the Flour. *Journal of Food and Nutrition Sciences*. **3(4),** pp. 147-151, (2015).

[13]. YUSUFU, P.A., NETALA, J. & OPEGA, J.L. Chemical, Sensory and Microbiological Properties of Cookies Produced from Maize, African Yam Bean and Plantain Composite Flour. *Indian J Nutri*.; **3(1),** 122, (2016).

[12]. OKOYE, J.I. & OBI, C.D. Chemical Composition and Sensory Properties of Wheat-African Yam Bean Composite Flour cookies. Discourse Journal of Agriculture and Food Sciences, Vol. 5(2): 21-27, (2017).

[14]. OMODARA T. R & ADERIBIGBE E. Y. Effects of the use of starter culture on the quality of fermented parkia biglobosa, International Journal of Bio-Technology and Research (IJBTR), Vol. 3, Issue 4, 33-40, (2013).

[15]. AOAC International. Official methods of analysis, 20th edn. (On-line). AOAC International, Rockville, MD (2016).

[16]. OLAGUNJU, O.F., EZEKIEL, O.O., OGUNSHE, A.O., OYEYINKA, S.A. & IJABADENIYI, O.A. Effects of fermentation on proximate composition, mineral profile and

antinutrients of tamarind (Tamarindus indica L.) seed in the production of daddawa-type condiment. *LWT - Food Science and Technology,* **90**, 455-459, (2018).
[17]. OJINNAKA, M.C. & OJIMELUKWE, P.C. Study of the volatile compounds and amino acid profile of *Bacillus* fermented castor oil bean condiment. *Journal of Food Research,* **29(1),** 192-203, (2012).
[18]. CHANDRA, S. & SAMSHER S. Assessment of functional properties of different flours. *African J. of agric*, 8(38): 4849-4852, (2013).
[19]. STONE H., BLEIBAUM R.N., & THOMAS H.A. Affective testing. In: Stone H., Bleibaum R.N., & Sidel J.L. (eds) Sensory evaluation practices, 4th edn. Elsevier Academic Press, pp 306–309. (2012).
[20]. EZEKIEL, O.O., OGUNSHE, A.A., & JEGEDE, D.E. Controlled fermentation of cotton seeds *(Gossypium hirsutum)* for Owoh production using starter cultures. *Nig. Food J.,* **33**, 54-60, (2015).
[21]. CHUKWU, M., NWAKODO, C.S., NDULAKA, J.C. & NWOKOCHA, N.J. Production and Proximate Composition of Ogiri-Ahuekere (Arachis hypogaea Linn) Seed Condiment. Research *Journal of Agriculture and Environmental Management*, **7(1):** 007-017, (2018).
[22]. AHAOTU, N.N., ECHETA, C.K., BEDE, N.E., AWUCHI, C.G., ANOSIKE, C.L., IBEABUCHI, C.J., & OJUKWU, M. Study on the nutritional and chemical composition of "Ogiri" condiment made from sandbox seed (*Hura crepitans*) as affected by fermentation time. *GSC Biological and Pharmaceutical Sciences*, ***11*(2),** 105–113, (2020). https://doi.org/10.30574/gscbps.2020.11.2.0115
[23]. DIETHER, N.E. & WILLING, B.P. Microbial Fermentation of Dietary Protein: An Important Factor in Diet–Microbe–Host Interaction. *Microorganisms*, ***7*(1):** 19, (2019). https://doi.org/10.3390/microorganisms7010019
[24]. ODUNFA, S.A. Microbiological and toxicological aspects of fermentation of castor oil seeds for *ogiri* production. *Journal of Food Science*, **50(6),** 1758-1759, (1981).
[25]. ADEBIYI J.A., OBADINA A.O., ADEBO O.A. & KAYITESI E. Comparison of nutritional quality and sensory acceptability of biscuits obtained from native, fermented, and malted pearl millet (Pennisetum glaucum) flour, *Food Chemistry*, **232**, 210-217, (2017).
[26]. DAKWA, S., SAKYI-DAWSON, T., DIAKO, C., ANNAN, N.T., & AMOA-AWUA, W.K. Effects of boiling and roasting on the fermentation of Soybean into Dawa Dawa (Soy-Dawa Dawa). *Int. J. Food Microbiol.* **10:** 69-82, (2005).
[27]. OLUSEYI, E.O. & TEMITAYO, O.M. "Chemical and functional properties of fermented, roasted and germinated tamarind (Tamarindus indica) seed flours", Nutrition & Food Science, Vol. 45 No. 1, pp. 97-111, (2015).
[28]. ADEBIYI, J.A., NJOBEH, P.B. & KAYITESI, E. Assessment of nutritional and phytochemical quality of Dawadawa (an African fermented condiment) produced from Bambara groundnut (*Vigna subterranea*), *Microchemical Journal,* **149:** 104034, (2019). https://doi.org/10.1016/j.microc.2019.104034.
[29]. NWOKELEME, C. O., & UGWUANYI, J. O. Evolution of Volatile Flavour Compounds during Fermentation of African Oil Bean (Pentaclethra macrophyllaBenth) Seeds for "Ugba" Production. International Journal of Food Science, 2015, 1–8, (2015).
[30]. TULEUN, C.D., CAREW, S.N. & PATRICK, J.A. Proximate and anti-nutritional factors profile of some varieties of velvet beans (*Mucuna spp*) found in Benue State of Nigeria. *Proceedings of the 13th Annual conference of Animal Science Association of Nigeria (ASAN), ABU, Zaria.* Pp. 550-553, (2008).
[31]. AKINMUTIMI, A.H. Effect of cooking period on the nutrient composition of velvet beans (*Mucuna pruriens*). *Proceedings from the 32nd Annual Conference of Nigeria Society for Animal Production, University of Calabar*. Pp. 223-236, (2007).