Nutritive and Phytate Contents of Raw and Fermented African Oil Bean Seeds

Obianom, A.O, Odibo, F.J.C, Iheukwumere, I.H

Nnamdi Azikiwe University, Awka, Nigeria

DOI: https://doi.org/10.51584/IJRIAS.2024.905036

Received: 26 April 2024; Revised: 11 May 2024; Accepted: 16 May 2024; Published: 18 June 2024

ABSTRACT

Studies have shown that consumption of plant foods that contain high amount of phytate (phytic acid) is associated with decrease in nutrient absorption, mineral deficiency and low sperm count. Producing natural phytase without any health or environmental challenge targeted the main focus of many researchers. This study was undertaken to evaluate the nutritive and phytate contents of raw and fermented African Oil bean seeds (*Pentaclethra macrophylla*). Prepared raw and fermented Oil bean seeds were randomly purchased from various markets in Awka metropolis, Anambra State, Nigeria, and their nutritive and phytic acid contents were determined using gravimetric and instrumentation techniques. The fermented PM seeds were screened for phytase-producing bacteria using standard plate technique. The data generated from the study were analyzed statistically using one way Analysis of Variance (ANOVA) and Student's "t" test. The study revealed significant ($P < 0.05$) reduction in carbohydrate content (23.82±0.14, 2.12±0.01) non-significant ($P > 0.05$) reduction in ash (0.54±0.01, 0.17±0.00) and fiber contents (4.88±0.07, 1.33±0.01) increase in moisture (30.40±0.03, 42.13±0.11) and protein contents (17.84±0.21, 22.97±0.11) and significant ($P < 0.05$) reduction in phytic acid (26.48±0.14, 0.02±0.00) during fermentation. Therefore, this study has shown that seeds of Oil bean contain vital nutrients, and phytate, which decreased during fermentation.

INTRODUCTION

Pentaclethra macrophylla (Oil bean) is a tropical tree crop which is commonly grown in Southern and Eastern Nigeria, and some part of West and Central Africa that are rain forest zones (Asad *et al*., 2022). The plant is a legume, and is generally recognized by peasant farmers as highly essential in nutrient improvement, and in agro-forestry system (Samtiya *et al*., 2020). *P. macrophylla* had been recognized as a food tree species for disbursing farms in the forest zone.

The edible seeds of the oil bean need rigorous but careful processing, and fermentation prior to consumption as food supplement (Roy *et al.*, 2014). Some parts of the plant have medicinal values, and several cases of ailment had been tackled such as convulsion and infertility (Asad *et al*., 2022). The fermented seeds are used traditionally as condiments and seasonings to add flavour to food (Zhu *et al*., 2014) and used for the preparation of many delicious African delicacies and snacks including, African salad, soups and sausages for eating with different staples. It is rich in vitamins and mineral and in high demand for both local consumption and for export as documented by several researchers (Zhuo *et al.*, 2014; Zhu *et al*., 2014; Asad *et al*., 2022). The seeds of oil bean which are rich in nutrients (protein, fatty acids and minerals have been found to be a highly nutritive animal feed when fortified (Dan *et al*., 2017).

The incidence of protein deficiency is high especially among low income earners because they cannot afford meat, fish, and other proteinous animal products due to their exorbitant costs, but frequent intake of fermented oil bean seed had contributed immensely in ameliorating the menace (Klosowski *et al*., 2018)

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume IX Issue V May 2024

Research had shown that oil bean seed contains anti-nutritive compound known as phytate, which binds to minerals, thereby preventing their absorption (Kammoun *et al*., 2012). The presence of phytate in oil bean makes it undesirable, but the ability of an enzyme known as phytase to breakdown the anti-nutritive compound, and restore the nutrient absorption had been documented (Roy *et al*., 2014; Kammoun *et al*., 2012; Asad *et al*., 2022).

Several researchers had worked on nutritive and phytate contents of fermented African Oil bean seed such as Gessler *et al*. (2018), Samtiya *et al*. (2020) and Liu *et al*. (2022) but little research had been documented on the nutritive and phytate contents of raw and fermented African Oil bean seeds. Hence, the aim of this study is to evaluate the nutritive and phytate contents of raw and fermented African Oil bean seeds. The result obtained in this study would provide indebt knowledge on the best form in which Oil bean seed could be consumed for an optimum health benefits.

MATERIALS AND METHODS

Sample Collection: This was carried out using the modified method of Suleiman and Omafe (2013). Prepared raw and fermented African Oil Bean (AOB) seeds were randomly purchased from different shops and open markets in Eke Awka market, Anambra State. Sampling was performed manually from different trays and basins. The samples were aseptically packed in sterile nylon bag; each sample was properly labeled and taken to the laboratory for analysis.

Transportation: A sterile polythene bag containing ice blocks placed inside a cooler was used for the transportation of the sample. The temperature of the cooler was carefully checked and adjusted to 28^0C -30^0 C in order to prevent or reduce microbial shock by reducing the quality of the ice inside the cooler. The samples were aseptically arranged inside the cooler without direct contact with the ice bag. The cooler was covered properly with packing tape to prevent accidental opening of the cooler. The cooler was taken to the laboratory safely for the analysis.

Nutritional content: The nutritive constituents of the raw and fermented African Oil Bean seed sample were determined using the method of AOAC (2012)

Moisture content: A crucible was dried and cooled, then initial weight of the crucible was taken as W_1, 10 g of the sample was transferred into the crucible and the weight of the crucible was taken W_2. The crucible and its content were then heated in an oven at 105^{o}C for 4-6 h. After drying the final weight of the crucible and its content were taken as W_3. Then the percentage moisture content was calculated as follows:

% moisture content = $(W_2 – W_3) / (W_2 –W_1)$ x 100/1

Ash Content: A crucible was dried and cooled, then initial weight of the crucible was taken as W_1, 10 g of the sample was transferred into the crucible and the weight of the crucible was taken W_2 .The crucible and its content were then heated in a furnace at 550^{o}C for 3-5 h, after which the crucible was removed and allowed to cool. The final weight of the crucible and its content was taken after drying/ashing as W_3. The percentage ash content was then calculated as follows:

% Ash content = $(W_3 –W_1)/ (W_2 – W_1)$ x100/1

Fats: A soxhlet extractor was used, the soxhlet flask was dried in an oven at 105^{o}C and allowed to cool, after which the weight of the flask was taken as W_1. Then 10 g of the sample was taken as W_2 and transferred into the thimble of the extractor, the sample was extracted using 250 mL of hexane for 4-5 h. After 5 h the chaff was emptied properly for the determination of the fibre content and all the solvent were recovered. The flask that contains all the extract were dried in an oven at 105^{O}C until all the water have

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume IX Issue V May 2024

been evaporated leaving the oil only, the weight of the flask and oil content was taken as W3. The percentage fat was calculated as follows:

% Fat = $(W_3 - W_1)/ W_2$ x 100/1

Fiber content: The residue was then transferred into a beaker and boiled for another 30 minutes with 200 mL of dilute sodium hydroxide solution and filtered, transferred into an ignited crucible.

The residue was then washed 3 times with 20 ml ethanol and 2 times with 10 mL petroleum ether. The residue was dried in an oven and cooled, then weighed (w_2). The dried residue was transferred into a furnace and ignited, cooled and weighed (w_3).

Calculation

Percentage Crude Fibre = $(w_2-w_3)/w_1$ x 100/1

Protein: A 0.5 g of the sample was weight and transferred into a digestible flask, 20 mL of sulphuric acid and 0.5 g of selenium powder (catalyst) were added, this mixture were heated in a fume cardboard for about 7 h (i.e until a clear or colourless solution is seen). The sample generated was diluted (1:4 dilution was carried out), 5 mL of the diluted sample was collected into a distillation flask and 5 mL of 40 % NaOH was added,then 10 mL of 10 % boric acid was put inside a conical flask, five drops of bromocresol green and 1drop of methyl red was also added and was properly mixed. The conical flask was placed under the tip of the condenser and the distillation started, 50 drops of the distillate was allowed to enter into the conical and then the color of the solution was turned blue. A burette was filled with 0.01 HCl and titrated against the content of the flask until the colour was changed to wine red, the titrate value was taken

% Nitrogen = titre x molarity of acid used (0.01M) x atomic mass of nitrogen x DF

=Tv x M x A x DF

Carbohydrate content: This can be calculated from the five other parameters as follows

Carbohydrate = 100 – (proteins + fats + fiber + ash + moisture)

Phytic acid content: This was determined using the method described in the study published by Adeyemo and Onilude (2013). Two grams of each sample was weighed into 250 ml conical flask (Pyrex). These were soaked in 100 ml of 2 % hydro chloric acid (HCL) and allowed for 3 h. These were filtered through double layered filter paper (What man NO.1). The filtrates were each placed in 250ml beaker (Pyrex) and added 100 ml of distilled water. Then 10ml of 0.30 % ammonium thiocyanate solution was added into each solution as indicator. This was titrated with standard iron (III) chloride solution, which contained 0.0019 g iron per ml. The end point was slightly brownish yellow, which persisted for 5 min. The percentage phytates for each sample was calculated using the formula

% Phytates = (X × 1.19 x 100)/ 0.00195

Where X = Titre Value

Screening for Phytase Producing Bacterial Isolates from Fermented African Oil Bean Seeds

This was carried out using the method described in the study published by Akter *et al*. (2018), Kalsi *et al.* (2016), Mogal *et al*. (2017) and Matrol *et al*. (2023).

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume IX Issue V May 2024

Qualitative determination of phytase: Ten grams of fermented Pentaclethra macrophylla seeds was weighed into 250 mL of conical flask (Pyrex), about 40 mL of normal saline (0.85% NaCl). This was shake thoroughly and then made up to 100 mL using the normal saline. Then 0.1 mL of this suspension was plated onto phytase screening medium (PSM) (1.5%glucose, 0.5% (NH4)2SO4, 0.05% KCl, 0.01% MgSO4.7H2O, 0.01% NaCl, 0.01% CaCl2.2H2 O, 0.001% FeSO4, 0.001% MnSO4, 3.5 % of agar agar, pH 6.5 with 0.5% sodium phytate (Sigma)). The colonies exhibiting zones of clearance (translucent areas) were selected and streaked on phytase screening medium containing sodium phytate as substrate. The clearance zone and colony diameter were measured after 2-5 days of incubation at 37°C. The phytase index was determined as follows:

Phytase Index = (Diameter zone of inhibition – Diameter of the colony) / Diameter of the colony

Characterization of the Selected Bacterial Isolates

The pure isolates were characterized using the morphological, biochemical and molecular characteristics as described by Iheukwumere *et al.* (2018).

Morphological characteristics of the bacteria isolates: The cultural descriptions (size, appearance, edge, elevation, colour) of the isolates were carried out. The Gram staining technique which revealed the Gram reaction, cell morphology and cell arrangement was carried out using the procedure described in the study published by Iheukwumere *et al.* (2018).

Gram staining technique: A thin smear was made in a cleaned grease free microscopic slide (75 mm×25 mm), air dried heat fixed. The smear was flooded with crystal violet solution (0.2 %) for 60 seconds and rinsed with cleaned water. Gram iodine solution (0.01 %) was then applied and allowed for 60 seconds. This was rinsed with cleaned water. This was followed by decolourizing the slide content with 95 %w/v ethyl alcohol for 10 seconds and then rinsed with cleaned water. The smear was then counter stained with safranin solution (0.025%) for 60 seconds, rinsed with cleaned water, blot drained and air dried. The stained smear was covered with a drop of immersion oil and observed under a binocular compound light microscope using × 100 objective lens.

Motility test: A semi-solid medium prepared by mixing 5.0 g of bacteriological agar (BIOTECH) with 2.0 g of nutrient broth (BIOTECH) in 1 Litre of distilled water was used. The solution was dissolved and sterilized using autoclaving technique after dispensing 10 mL portion in different test tubes. The test tubes were allowed to set in vertical positions, and the test organisms were then inoculated by performing a single stab down the centre of the test tube to half the depth of the medium using sterile stabbing needle. The test tubes were kept in an incubator in vertical position at 352^{o}C for 24 h.

Biochemical characteristics of the isolates: The capability of the isolates to produce catalase, indole, oxidase, acetoin, and to utilize sugars, sugar alcohols was done using the methods described in the study published by Iheukwumere *et al.* (2018).

Indole test: Indole is a nitrogen containing compound formed when the amino acid tryptophan is hydrolyzed by bacteria that have the enzyme tryptophanase. This is detected by using KOVAC's reagent. For this test, isolates were cultured in peptone water in 500.0 mL of deionized water. Ten millilitres of peptone water was dispensed into the test tubes and sterilized. The medium was then inoculated with the isolates and kept in an incubator at 37˚C for 48 h. Five drops of KOVAC's reagent was carefully layered onto the top of 24 h old pure cultures. The presence of indole was revealed by the development of red layer colouration on the top of the broth cultures.

Sugar fermentation test: The capability of the isolates to metabolize some sugars with the resulting

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume IX Issue V May 2024

formation of acid and gas or either was carried out using sugar fermentation test. One litre of 1 % (w /v) peptone water was added to 3 mL of 0.2 % (w/v) bromocresol purple and 9 mL was dispensed in the test tube that contained inverted Durham tubes. The medium was then sterilized by autoclaving. The sugar solution was prepared at 10 % (w/v) and sterilized. One milliliter of the sugar was dispensed aseptically into the test tubes. The medium was then inoculated with the appropriate isolates and the cultures incubated at 37˚C for 48 h and was examined for the formation of acid and gas. Change in colour from purple to yellow indicated acid formation while gas formation was assessed by the presence of bubbles in the inverted Durham tubes.

Hydrogen sulphide production: This was performed using triple sugar iron (TSI) agar. The TSI agar was made in accordance to the manufacturer's instruction. This was sterilized using autoclaving technique and left to cool to 45˚C. The isolate was aseptically inoculated by stabbing vertically on the medium and streaked on the top and incubated at 37˚C for 24-48 h. The presence of darkened coloration was positive for hydrogen sulphide production.

Methyl red test: The glucose phosphate broth was prepared according to the manufacturer's direction and the isolates were aseptically inoculated into the sterilized medium. This was incubated at 37˚C for 48 h. After incubation, five drops of 0.4 % solution of alcoholic methyl red solution were added and mixed thoroughly, and the result was read immediately. Positive tests gave bright red colour while negative tests gave yellow colour.

Voges-Proskauer test: The glucose phosphate broth was prepared in accordance to the manufacturer's direction, and the isolates were aseptically inoculated into the sterilized medium. This was incubated at 37˚C for 48 h. After incubation, 1.0 mL of 40 % potassium hydroxide (KOH) containing 0.3 % Creatine and 3 mL of 5 % solution of α-naphthol was added in the absolute alcohol. Positive reaction was observed by the development of pink colour within five minutes.

Citrate utilization test: The Simmon's Citrate Agar was prepared according to the manufacturer's direction, and the isolates were inoculated by stabbing directly at the center of the medium in the test tubes, and incubated at 37˚C for 48 h. Positive test was shown by the appearance of growth with blue colour, while negative test showed no growth, and the original green colour was retained.

Catalase test: A smear of the isolate was made on a cleaned grease-free microscopic slide. Then, a drop of 30 % hydrogen peroxide (H_2O_2) was added on the smear. Prompt effervescence indicated catalase production.

Oxidase test: The test involved two drops of freshly prepared oxidase reagent that was dispensed on Whatman No. 1 filter paper which was placed in Petri dish, and a smear of the test isolate was made on the spot using a sterile stick. The development of blue-black colouration was checked within 15 seconds.

Urease test: The urea agar slant was prepared in accordance to the manufacturer's direction and the isolates were aseptically inoculated into sterilized medium. This was incubated at 37^{o}C for 48 h. After incubation, observation was made for the presence of purple-pink colouration.

Gelatin hydrolysis test: Gelatin agar (BIOTECH) was used for this test. The medium was prepared according to the manufacturer's direction and poured into Petri dishes and allowed to solidify. The test isolates was aseptically streaked on the surface of the medium, and then incubated for 24-48 h at 35±2^{o}C. The hydrolysis of the medium was checked after 24-48 h incubation.

Starch hydrolysis: This is used to determine the ability of the bacteria to hydrolyze starch. The starch agar was prepared in accordance to the manufacturer's direction, and the isolates was aseptically streaked into sterilized medium and incubated for 48 h at 37^{o}C, the surface of the plate was flooded with iodine solution

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume IX Issue V May 2024

with a dropper for 30 sec., excess iodine was poured off, presence of clear zone around the line of bacterial growth indicates positive results while a blue, purple or black coloration of the medium indicated negative result.

Data Analysis

The data obtained in this study were presented in Tables. One way Analysis of Variance was used to determine the significance of the sample sources 95% confidence level. Pairwise comparison was carried out using student "t" test (Iheukwumere *et al*. 2018).

RESULTS AND DISCUSSION

The nutritive content of the studied *Pentaclethra macrophylla* (African Oil bean) seeds are shown in Table 1. The study revealed significant ($P < 0.05$) reduction in the carbohydrate content during fermentation, and this was seen most after 96 h fermentation. There was also reduction in ash content and crude fibre contents in every 24 h intervals. The moisture content also showed increase in every 24 h intervals including the fat contents which showed significant ($P < 0.05$) increase after 96 h. There was slight increase in the protein content in every 24 h intervals. The phytate content of the raw and fermented African Oil bean seeds are shown in Table 2. The study revealed significant ($P < 0.05$) reduction in the phytate content in every 24 h intervals from 26.48 mg/100 g to 0.02 mg/100 mg after 96 h.

Table 1: Nutritional parameters of African Oil bean seeds during fermentation

Parameter (%)	Raw	24 h	48 h	72 h	96 h
Moisture	30.40±0,03	36.34±0.11	38.86±0.07	41.48±0.14	42.13±0.11
*Carbohydrate	23.82±0.14	13.83±0.14	8.15±0.07	4.03±0.03	2.12±0.01
Protein	17.84±0.21	19.22±0.12	21.94±0.11	22.61±0.21	22.97±0.11
Fat	22.52±0.11	26.61±0.42	28.52±0.11	29.86±0.17	*31.28±0.22
Crude Fibre	4.88±0.07	3.67±0.01	2.27±0.01	1.84±0.01	1.33±0.01
Ash	0.54±0.01	0.33±0.00	0.26±0.00	0.18±0.00	0.17±0.00

*——Significance difference ($P < 0.05$)

Table 2: Phytate content of African Oil bean seeds during fermentation

Time (h)	*Phytate (mg/100 g)
0	26.48±0.14
24	8.42±0.11
48	1.18±0.01
72	0.12±0.00
96	0.02±0.00

Screening for Phytase-Producing Bacterial Isolates from Fermented African Oil Bean

Bacterial isolates that produced phytase from the studied fermented oil bean seeds are shown in Tables 3 and 4. The study revealed that isolates A3, A5Y1, B2Y2, B4, MO1, and MO2 were positive among the bacteria isolated from fermented oil bean seeds for phytase production (Table 3). Their clear diameter zones that indicated production of phytase were classified as small, moderate, and large as shown in Table 3. The diameter of the colonies that grew in Phytase Screening Medium (PSM), clear zones diameter and Phytase

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume IX Issue V May 2024

Index (PI) were determined and recorded as shown in Table 4. The study revealed that isolate MO1 recorded the highest PI, followed by B2Y2, B4, MO2, A5Y1, and A3 recorded the least value.

Table 3: Extracellular phytase producing bacterial isolates from fermented African oil beans seeds

Isolate Code	Inference	Size of Inhibitory Zone
A3	Positive	Small
A5Y1	Positive	Small
B2Y2	Positive	Large
B4	Positive	Moderate
MO1	Positive	Large
MO2	Positive	Moderate

Table 4: Qualitative determination of phytase producing bacterial isolates from the fermented samples

Isolate Code	Diameter of colony (cm)	Diameter of the clear zone (cm)	Phytase Index (PI)
A3	1.40	1.60	0.14
A5Y1	1.70	2.10	0.24
B2Y2	1.80	2.60	0.44
B4	1.60	2.20	0.38
MO1	2.00	3.00	0.50
MO2	1.70	2.20	0.29

Characteristics of Phytase-producing Bacterial Isolates

The cultural and morphological characteristics of the two most phytase-producing isolates revealed similar features; the similar size, irregular margin, opaque, flat, Gram positive rods, motile with production of endospores but differed on the appearance of their colonies on Nutrient Agar (NA). Isolate MO1 showed fuzzy white appearance whereas isolate B2 showed cream appearance. Isolate MO1 showed rough surface with round shape whereas isolate B2 showed shiny and moist surface which later became rough with hair-like growth shape. The position of endospore produced by isolate MO1 was sub-terminal whereas that of B2 was central. The cultural and morphological characteristics of isolates MO1 and B2 revealed that the isolates were *Bacillus* species (Table 5).

The biochemical characteristics of isolates MO1 and B2 showed that the isolates were positive to gelatin hydrolysis, catalase, Voges Prokauers, citrate, nitrate reductase, starch hydrolysis, glucose, arabinose, D-mannitol, maltose, and tetrahalose utilization, but negative to oxidase, methyl red, indole and urease. They varied in their utilization of galactose, sorbitol, dulcitol, and xylitol shown in Table 6.

Table 5: Cultural and morphological characteristics of the bacterial isolates

Parameter	Isolate MOI	Isolate B2
Colour of the colony on NA	Fuzzy white	Cream
Colony Size	Medium	Medium
Margin of Colony	Irregular	Irregular
Optical Nature	Opaque	Opaque
Elevation	Flat	Flat
Surface of the Colony	Rough	Shiny and moist, rough later

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume IX Issue V May 2024

Shape of the Colony	Round	Hair-like growth
Gram Reaction	Positive	Positive
Morphology	Rods	Rods
Endospore	Present	Present
Position of Spore	Sub-terminal	Central
Motility	Yes	Yes
Bacterium	*Bacillus* species	*Bacillus* species

Table 6: Biochemical characteristics of the bacterial isolates

Parameter	Isolate MO1	Isolate B2
Gelatin	+	+
Oxidase	_	_
Catalase	+	+
Methyl red	_	_
Voges Prokauer	+	+
Indole	_	_
Citrate	+	+
Urease	_	_
Nitrate reductase	+	+
Starch	+	+
D-glucose	+	+
Arabinose	+	+
D-mannitol	+	+
Maltose	+	+
Trehalose	+	+
Galactose	+	+
Sorbitol	+/_	+
Dulcitol	+	+/_
Xylitol	+/_	+/_
Bacterium	*Bacillus* species	*Bacillus* species

The presence of proteins, carbohydrates, fats, fibers, ash, and moisture in *Pentaclethra macrophylla* (Oil bean) seeds agrees with the findings of many researchers (Ogueke *et al*., 2010; Alinnor and Oze, 2011; Balogun, 2013; Okorie and Olasupo, 2013; Omeh *et al*., 2014; Oyekele *et al*., 2014; Nwokeleme and Ugwuanyi, 2015; Anioke, 2019; Duru *et al*., 2020). The significant decreased in carbohydrate content of the African Oil bean seeds during fermentation agrees with the findings of many researchers (Chelule *et al*., 2010; Subramaniyam and Vimala, 2012; Okorie and Olasupo, 2013; Eze *et al*., 2014) but agrees with the report of Ikhuoria *et al*. (2008) who observed slight decreased in the carbohydrate content. This slight variation could be attributed to poor calibration of instruction, poor measurement and technical skills associated with the researcher. There were also increased in the moisture content and proteins of the African Oil bean seeds during fermentation, and these were also reported by many researchers (Ogueke *et al*., 2010; Alinnor and Oze, 2011; Balogun, 2013; Okorie and Olasupo, 2013; Omeh *et al*., 2014; Oyekele *et al*., 2014; Nwokeleme and Ugwuanyi, 2015; Anioke, 2019; Duru *et al*., 2020) but these were disagreed by Igwenyi *et*

al. (2015), Aladekoyi *et al.* (2017) and Akinlabu *et al*. (2019) who reported slight decreased in protein content of African Oil bean seeds during fermentation.

The significant reduction in phytate content of African Oil bean seeds during fermentation to very minimal value as seen in the present study agrees with the findings of many researchers (Ogueke *et al*., 2010; Alinnor and Oze, 2011; Balogun, 2013; Okorie and Olasupo, 2013; Omeh *et al*., 2014; Oyekele *et al*., 2014; Nwokeleme and Ugwuanyi, 2015; Anioke, 2019; Duru *et al*., 2020). The phytases (myo-inositol hexakisphosphate phosphohydrolase) catalyze the hydrolysis of phytate (myo-inositol hexakisphosphate) to inorganic phosphate and less-phosphorylated myo-inositol derivatives. This decreased in phytate (phytic acid) content during fermentation could be attributed to the phytase secreted by bacterial organisms found in the fermentation African Oil bean seeds. The significant zones of inhibition as an indication around the bacterial isolates as indication of phytase production supported the findings of many researchers (Eze *et al*., 2014; Oyekele *et al*., 2014; Igwenyi *et al*.. 2015; Anioke, 2019) who screened for the presence of phytase-producing bacterial from different samples. The highest occurrence of phytase index (PI) associated with bacterial isolate MO1 in the study is an indication that the isolate has the ability to secrete enough phytase that can induce hydrolysis of phytate more than other bacterial isolates that were phytase positive. Eze *et al*. (2014) and Wulandari *et al*. (2015) reported the occurrences of high PI isolate in their studies.

The cultural, morphological, biochemical and molecular characteristics of the best phytase-producing bacterial isolates in the present study revealed *Bacillus subtilis* strain JD-014 (BS014) and *Bacillus licheniformis* strain CP13 (BLC13) for MO1 and B2 isolates. Many researchers (Alinnor and Oze, 2011; Balogun, 2013; Okorie and Olasupo, 2013; Eze *et al*., 2014; Oyeleke *et al.,* 2014; Igwenyi *et al*., 2015; Anioke, 2019) reported secretion and highest production of phytase by *Bacillus* species, and majorities of the researchers pointed *Bacillus subtilis* as the major producer of phytase.

CONCLUSION

The study has shown that *P. macrophylla* (African Oil bean) contains nutrients such as carbohydrates, proteins, vitamins, fats, and fibre. The nutritional parameters evaluated increased as the fermentation time increased. The Oil bean seeds contained phytic acid, which decreased as fermentation time increased and the bacterial species that was identified as phytase producer was *Bacillus subtilis*.

REFFERENCES

1. Akter, D., Khan, M., Mian, M., Khan, S.N. and Hoq, M. (2018). Phytases production from a novel Klebsiella sp. on wheat bran for animal feed digestion. *Microbial Bioactives* 7: 14 – 2
2. Akinlabu, D.K., Owolabi, F.E., Audu, O.Y., Ajanaku, C.O., Falope, F. and Ajani, O.O. (2019). Phytochemical and Proximate Analysis of African Oil Bean (*Pentaclethra macrophylla* Benth) Seed. *Journal of Physics* 1378: 32 – 57
3. Aladekoyi G., Orungbemi, O.O., Karim, O.A. and Aladejimokun, A.O. (2017). Comparative studies of the nutritional and phytochemical constituents of African oil bean (*Pentaclethra macrophylla* benth) and African bean (*Anthonotha macrophylla*) for human consumption. *Chemical Research Journal* 2(3): 16 – 21.
4. Alinnor, I. J. and Oze, R. (2011). Chemical Evaluation of the Nutritive Value of *Pentaclethramacrophylla* (African Oil Bean) seed. *Pakitan Journal of nutrition* 10: 355 – 359.
5. Anioke, I. C. (2019). Effect of Fermented *Pentaclethra Macrophylla* Benth (African Oil Bean) Seed Extract on Plasma Lipid Profile in Healthy Rat Model-A Preliminary Study. *South Asian Research Journal of Natural Products* 2(1): 1 – 9.
6. Asad, M.J., Ahmad, M.S. and Ahmad, T. (2022). Optimization of Phytase Production by *Bacillus* (HCYL03) under Solid-State Fermentation by Using Box–Behnken Design Rozina Sardar. *Brazilian*

Archives of Biology and Technology 65: 222 – 307

7. Balogun, B.I. (2013). Evaluation of the Nutritional Potentials of Fermented Oil Beans Seed *Pentaclethra macrophylla Production Architecture Technology* 9 (2): 73-87
8. Dan, S., K., Banerjee, G., Nandi, A. and Ray, A.K. (2017). Nutritional evaluation of soybean meal after fermentation with two fish gut bacterial strains, *Bacillus cereus* LRF5 and *Staphylococcus caprae* CCF2 in formulated diets for *Labeo rohita Journal of Fisheries* 5(1): 445 – 454
9. Duru, F.C., Ohaegbunam, P. and Nwachukwu, C.A. (2020). The proximate and anti -nutrient composition of African oil bean (*Pentaclethra macrophylla* Benth) Seed at different maturation stages and boiling periods. *Journal of Nutrition* 5: 79 – 89
10. 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(5): 138 – 142
11. Gessler, N., Serdyuk, E., Isakova, E. and Deryabina, Y.I. (2018). Phytases and the prospects for their application. *Applied Biochemistry and Microbiology* 54(4):352–360
12. Igwenyi, I.O., Isiguzo, O.E., Aja, P.M., Ugwu Okechukwu, P.C., Ezeani, N.N. and Uraku, A.J. (2015). Proximate Composition, Mineral Content and Phytochemical Analysis of the African Oil Bean (*Pentaclethra macrophylla*) Seed. *American-Eurasian Journal of Agriculture & Environmental Science* 15(9):1873 – 1875.
13. Kammoun, R., Farhat, A., Chouayekh, H., Bouchaala, K. and Bejar, S. (2012). Phytase production by *Bacillus subtilis* US417 in submerged and solid state fermentations. *Annals in Microbiology* 62:155–164
14. Kłosowski, G., Mikulski, D. and Jankowiak, O. (2018). Extracellular phytase production by the wine yeast *cerevisiae* (Finarome Strain) during submerged fermentation. *Molecules* 23(4):848.
15. Kalsi, H.K., Singh, R., Dhaliwal, H.S. and Kumar, V. (2016). Phytases from *Enterobacter* and *Serratia* species with desirable characteristics for food and feed applications. *Biotechnology* 6(1):64 – 77
16. Liu, X., Han, R., Cao, Y., Turner BL, Ma LQ (2022) Enhancing phytate availability in soils and phytate-P acquisition by plants: a review. *Environmental Science and Technology* 56(13):9196–9219.
17. Matrol, Z.H., Gerami, M., Ghaedi, K. and Majidian, P. (2023). Extraction and Molecular evaluation of phytase-producing bacteria rom soil of Alfalfa and Clover fields of Isfahan. *Journal of Genetic Resources* **9** (1): 11 – 16
18. Mogal, C., Singh, D., Mehta, A., Ahmad, T. and Suthar, K. (2017). Isolation and biochemical characterization of phytase from different sources. *Journal of Applied Biotechnology and Bioengineering* ***2(4)***:152–155
19. Nwokeleme, C. O. and Ugwuanyi, J. O. (2015). Evolution of volatile flavour compounds during fermentation of African oil bean (*Pentaclethra macrophylla* Benth) seeds for Ugba production. *International Journal of Food Science* 2: 156 – 166
20. Omeh, Y. N., Garuba, O., Adiele, I. P. and Ejiofor, U. E. (2014). Some kidney function parameters of wistar albino rat fed *Pentaclethra macrophylla* seeds meal. *European Journal of Biotechnology and Bioscience* 2 (3): 17 – 20.
21. Ogueke, C. C., Nwosu, J. N., Owuamanam, C. I. and Iwouno, J. N. (2010). Ugba, the fermented African oil bean seed; its production, chemical composition, preservation, safety, and health benefits. *Pak Journal of Biological Science* 13(10): 389 – 496
22. Okorie, C. P. and Olasupo, N. A. (2013). Controlled fermentation and preservation of UGBA – an indigenous Nigerian fermented food. *SpringerPlus* 2: 470 – 478
23. Oyeleke, G.O., Odedeji, J.O., Ishola, A.D. and Afolabi, O.(2014). Phytochemical Screening and Nutritional Evaluation of African Oil Bean (*Pentaclethramacrophylla*) Seeds. *IOSR Journal of Environmental Science, Toxicology and Food Technology* 8(2): 14 – 17
24. Samtiya, M., Aluko, R.E. and Dhewa, T. (2020). Plant food anti-nutritional factors and their reduction strategies: an overview. *Food Production Process Nutrition* 2(1):6.

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume IX Issue V May 2024

25. Wulandari, R., Sari, E.N., Ratriyanto, A., Weldekiros, H. and Greiner, R. (2015). Phytase-producing bacteria from extreme regions in Indonesia. *Brazilian Archives in Bio Technology* 58(5):711–717.
26. Zhu, Y., Qiu, X., Ding, Q., Duan, M. and Wang, C. (2014). Combined effects of dietary phytaseand organic acid on growth and phosphorus utilization of juvenile yellow catfish *Pelteobagrus fulvidraco*. *Aquaculture* 430: 1–8.
27. Zhuo, L.C., Liu, K. and Lin, Y.H. (2014). Apparent digestibility of soybean meal and *Lactobacillus* fermented soybean meal in diets of grouper, *Epinephelus coioides*. *Aquaculture Research* 47: 1009–1012.