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Effect of Spices on the Microbial Load and Physicochemical Properties of Fermented *Pakia Biglobosa* Seeds

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ABSTRACT: Fermented *Parkia biglobosa* seeds (iru) contain high protein content, serving as a major source of protein in rural areas, among people who cannot afford animal proteins. Spices are known to possess health benefits in humans. Hence, this study assessed the impacts of spices such as alligator pepper, ginger, garlic, nutmeg, turmeric, grains of Selim, cocoplum and skinplum on the microbial population and physicochemical properties of fermented *Parkia biglobosa* seeds. A 30g of each spice was added to 300g of previously pressure cooked and dehulled locust beans in separate containers. Each was sterilized and inoculated with *Bacillus subtilis*. All the samples including control (naturally fermented *P. biglobosa* alone) were fermented at 37^0C for 36h. The microbial load, pH, total titratable acidity (TTA) and proximate analysis were determined. Commercially fermented iru had the highest microbial load of 5.40, with unfermented iru having the least load (4.78). Unfermented iru had the least pH (6.35), the pH of all fermented samples was significantly high (P=0.05) with values ranging from 7.27 to 8.29. TTA of all the samples varied with the highest value of 5.75 recorded in cocoplum fermented iru while Selim fermented iru had the least value (0.85). Protein and fat contents of all fermented samples significantly increased (P=0.05) while carbohydrate and crude fibre contents decreased. Selim fermented iru had the highest ash content of 6.07 while alligator fermented iru had the least ash content (2.86). This research confirmed that fermentation of *P. biglobosa* with edible spices improved its nutritional value in production of iru.

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Seeds of *Parkia biglobosa* possess high protein contents. In the Western part of Nigeria, seeds of *Parkia biglobosa* are usually fermented to produce 'iru', a local condiment used for cooking delicacies. (Omodara and Aderibigbe 2014). This fermented product is an alternative source of protein for low-income earners, whose protein intake is low due to continuous increase in the price of animal protein sources (Ojewumi *et al.*, 2016). The processing of locust bean seeds involves several stages which include cooking, dehulling, washing, fermenting, salting and refrigerating (Das et al. 2022). Apart from being rich in protein, there are some other health benefits of consuming fermented *Parkia biglobosa* seeds which include controlling diabetes and cholesterol level, promoting good sight, aiding in digestion, treating stroke and hypertension, reduction in blood sugar level, management of bacterial infections, treatment of diarrhea and also for enhancing weight loss (Saleh et al. 2021). In fermentation industries, microorganisms play major role in the production of some metabolites such as

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acids, alcohols, enzymes, antibiotics, carbohydrates. The consumption of live cells of desirable microorganisms and their metabolic products in fermented foods does not constitute any health hazard to the consumers (Voidarou et al. 2020). From previous research, the major fermenting microorganisms of *Parkia biglobosa* seeds are dominated by mixed bacterial populations which include strains of *Bacillus* group, *Staphylococcus epidermidis* and *Lactobacillus* species (Adelekan et al. 2017). To enhance the fermentation process and have standardized fermented products, with improved keeping qualities, few good strains of *Bacillus subtilis* group, have been used as starter cultures (Omodara and Aderibigbe 2014). The growths of these fermenting organisms are affected by some instrinct and extrinct factors such as pH, temperature, Total Titrable Acidity (TTA) and moisture contents (Mokoena et al., 2016).

Spices are strong smelling and sharp-tasting food substances usually used to improve or enhance the flavor of food. Spices can also be described as dried seeds, fruits, root, bark, leaves or vegetative substances used in small quantities as food additives for the purposes of flavor, colour, or as preservatives (Sachan et al 2018). They are usually of vegetable source, e.g., mustard, ginger, garlic, coriander, locust bean, etc. (Odebunmi et al., 2009). In order to enhance the health benefit of the fermented *Parkia biglobosa* seeds, like in management of bacterial infections, aiding digestion by boosting the gut microbes, treatment of diarrhea, enhancing weight loss and improvement of vision, there is need to fortify this fermented product with some spices so as to produce fermented products of better qualities. From the previous work, the microorganism responsible for fermentation has played major role by improving nutritional composition of the fermented product when compared with unfermented product. Therefore, the objective of this research is to evaluate the effect of spices on the microbial load and physicochemical properties of fermented *Pakia biglobosa* seeds.

MATERIALS AND METHODS

Source of materials: The African locust bean (*Parkia biglobosa*) seeds used for the research was purchased from Central market in Kota Ekiti, Ekiti State. The spices used were *Zingiber officinale* (ginger), *Allium sativum* (garlic), *Curcuma longa* (turmeric), *Myristca fragrans* (nutmeg), *Perinari excelsa* (skinned plum), *Chrysobalanus icaco* (Coco plum), *Aframomum melegueta* (Guinea pepper) and *Xylopia aethiopica* (selim). All the spices were purchased from Oja-Oba in Ado-Ekiti, Ekiti State. Pure culture of *B. subtilis* (strain 2B) was obtained from the stock cultures kept in the Department of Microbiology, Ekiti State University, Ado-Ekiti, Ekiti State. This strain had been previously used by Omodara and Aderibigbe (2014) to produce 'iru-woro' (the hard-type of fermented *Parkia bilobosa* seeds).

Preparation of starter culture: The starter culture was prepared by using the method of Omodara and Aderibigbe, (2014)

Preparation of the spices: Hundred grams (100g) of each spice was weighed and cleaned. Root of *Zingiber officinale* and *Curcuma longa* rhizome were washed, peeled and cut into smaller pieces. *Curcuma longa* rhizome was washed, peeled and cut into smaller pieces. *Myristca fragrans* was grated using grater. The seeds of *Perinari excelsa* were shelled. *Chrysobalanus icaco*, *Aframomum melegueta* and *Xylopia aethiopica* seeds were removed from the pod. The membranous skin of *Allium sativum* bulbs was removed and the cloves were cut into smaller pieces. All the samples were washed with sterile water, dried at 50^{o}C until a constant weight was archived and finely ground using blender. 30g of the blended spices was used for the research.

Laboratory production of iru: The method of Omodara and Aderibigbe (2014) was adopted. The seeds were soaked in water for 15 min, boiled under pressure (by using pressure pot for 2 h), dehulled by rubbing between palms to remove the testa. Three hundred grams (300 g) each of the cotyledons were weighed into nine different 1L-beakers. The 300 g cotyledons in first beaker was poured into pressure pot and boiled for 1 h, drained and aseptically poured into a sterile fermenting can of 10 cm × 20 cm × 10 cm rectangular-shaped aluminum fermenting can and was labeled as naturally fermented 'iru' (NFI). Thirty grams (30 g) each of finely ground spices were added separately to cotyledons in beakers 2, 3, 4, 5, 6, 7, 8 and 9. These were poured into separate pressure pots and boiled at 121°C for 1 h. After boiling, the boiled cotyledons were poured aseptically into different sterile fermenting cans of the same dimension used above and they were labeled as; UnFI (Unfermented iru), NaFI (Naturally fermented iru), StFI (Starter culture fermented iru), AiFI (Alligator fermented iru), GiFI (Ginger fermented iru), NuFI (Nutmeg fermented iru), GaFI (Galic fermented iru), TuFI (Tumeric fermented iru), GsFI (Grain of selime fermented iru), CoFI (Cocoplum fermented iru), SkFI (Skinplum fermented iru), CmFI (Commercially fermented iru). All the spiced samples were inoculated with 1.0 ml of the starter culture *B. subtilis* 2B and were fermented at 35°C for 36 h. The naturally fermented 'iru' NFI served as control.

Microbiological analysis: The microbial analysis carried out include isolation of microorganisms from the samples, determination of total viable counts (microbial load) using direct microscopic observation of the isolates (Olutiola et al., 1991).

pH determination: The method of AOAC (2000) was used to determine the pH of the samples. The extract was prepared by homogenizing 5.0g of each sample in 100ml distilled water. This was filtered using Whatman No 1 filter paper. The filtrate was used as extract. The pH was determined by pH meter (ELE Model No1) which was standardized using buffers at pH 4.0 and 9.0. The electrode was dipped into a beaker containing 20ml of the sample extract. The pH values were determined in triplicates.

Determination of TTA: The TTA of the fermented and unfermented samples were determined by the method of AOAC (2000). The extract was prepared by homogenizing 5.0g of each sample in 100ml distilled water. This was filtered using Whatman No 1 filter paper. The filtrate was used as extract. Then 20ml of the filtrate was titrated against 0.1M NaOH in a burette, using 2-3 drops of phenolphthalein as indicator. The TTA was calculated using the equation 1.

$$NaVa = NbVb \quad (1)$$

Moisture content determination: The method of AOAC 2000 was adopted in the determination of moisture content. The moisture contents of the fermented and unfermented samples were determined by the method of AOAC (2000). Five grams (5g) of each sample was weighed separately into a pre-weighed aluminum foil. These were put in oven at 105^{o}C for 3h and weighed intermittently until a constant weight was achieved. The new weight was subtracted from the weight of the wet sample. The percentage moisture content was calculated as equation 2

$$\text{Moisture content (\%)} = \frac{\text{WWS} - \text{WDS}}{(\text{WWS})} * 100 \quad (2)$$

Where WWS = weight of wet sample; WDS = eight of dry sample

Proximate analysis: The proximate compositions of the fermented samples were determined using standard procedures of AOAC (2000). The parameters determined were protein, ash, crude fibre, fat and their carbohydrate. The crude protein content was calculated by multiplying the total nitrogen with the factor 6.25, using Kjedahl method (Joslyn, 1970); and crude fibre by AOAC (2000). The amount of lipid (oil) was determined, using Soxhlet extraction method; while the ash content was determined by the method of AOAC (2000), and the carbohydrate content of each sample was determined by difference.

RESULTS AND DISCUSSION

Figure 1 showed the microbial load of the unfermented, starter culture fermented and spices fortified fermented *Parkia biglobosa* seeds (Log cfu/g). All the samples had significantly lower microbial load compared to the commercially fermented iru which had the highest microbial population of 5.4, while unfermented iru had the least microbial load (4.75). The pH of the unfermented 'iru' and spices fortified fermented 'iru' is presented in figure 2. All the samples including unfermented 'iru' had high pH values. Unfermented 'iru' (UnFI) had the least pH value of 6.35. The pH of GaFI, NuFI, GsFI, TuFI and AIFI are 6.95, 7.02, 7.24, 7,24 and 7.33 respectively, significantly lower than that of CmFI (7.6). However, StFI, GiFI, SlFI and CoFI had significantly higher pH of 7.96, 8.02, 8.03 and 8.27 respectively, when compared with the commercially prepared 'iru' (CmFI). It is important to note that NaFI and CmFI had same pH (7.6). Figure 3 showed the total titratable acidity (TTA) of the unfermented, starter culture fermented and spices fortified fermented *Parkia biglobosa* seeds. GsFI had the lowest TTA of 0.85, while CoFI had the highest TTA of 5.75. Commercially fermented *Parkia biglobosa* seeds had TTA value of 1.5 The percentage moisture content of the unfermented, starter culture fermented and spices fortified fermented *Parkia biglobosa* seeds is presented in figure 4. UnFI had the least moisture content (50%). The moisture content of CmFI and StFI were slightly higher at 54 and 55 respectively. All the other samples had significantly high moisture content, with the highest value recorded in CoFI (68%).

Table 1 showed the result of the proximate analysis of the unfermented, starter culture fermented and spices fortified fermented *Parkia biglobosa* seeds. All the fermented samples had significantly higher protein content compared to unfermented iru which had the least value of 20.19%. GiFI and StFI had the highest protein cotent of 41.85% and 41.22% respectively.

The ash content of commercially produced 'iru' was 3.99%. NaFI, AlFI, NuFI and TuFI had lower ash content of 3.79, 2.86, 2.95 and 3.79% respectively, compared to CmFI. However, UnFI, StFI, GaFI, GsFI, SkFI and GiFI had significantly higher percentage ash content (4.30, 5.04, 5.06, 6.07, 4.73 and 4.87% respectively).

UnFI had the highest crude fibre content (8.23%). CmFI, NaFI and NuFI had slightly high crude fibre content (6.03, 5.46, 5.46% respectively). All the spice-fortified locust beans had significantly low fibre content, except NuFI (5.46%). All fermented seeds had significantly higher fat content when compared unfermented sample. NaFI, GiFI and NuFI had relatively highest fat level (19.75, 19.75 and 19.50% respectively). All spiced condiments had higher fat level compared to CmFI (15.13%), except GsFI which had fat content of 14.05%. UnFI recorded the highest carbohydrate content (36.81). the carbohydrate content of NaFI, StFI, GiFI, NuFI and GaFI were significantly lower than that of CmFI. However, UnFI, AlFI, TuFI, GsFI, CoFI and SkFI had higher carbohydrate content of 36.81, 34.27, 35.78, 33.64, 31.80 and 35.24 respectively.

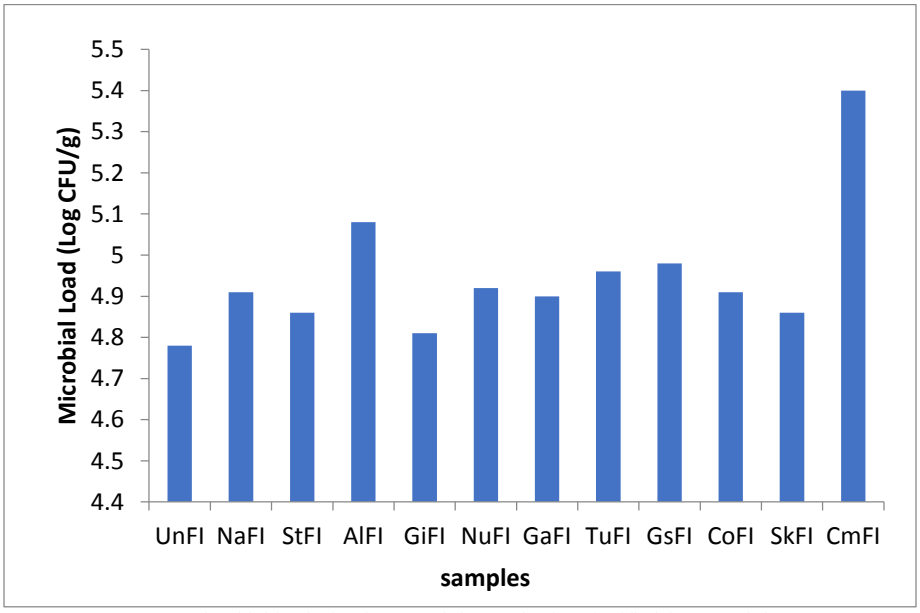

Fig 1: Microbial load of unfermented 'iru' and spices fortified fermented 'iru'

Key: UnFI = Unfermented iru, NaFI = Naturally fermented iru, StFI = Starter culture fermented iru, AiFI =Alligator fermented iru, GiFI = Ginger fermented iru, NuFI = Nutmeg fermented iru, GaFI = Galic fermented iru, TuFI = Tumeric fermented iru, GsFI = Grain of selime fermented iru, CoFI = Cocoplum fermented iru, SkFI = Skinplum fermented iru, CmFI = Commercially fermented iru.

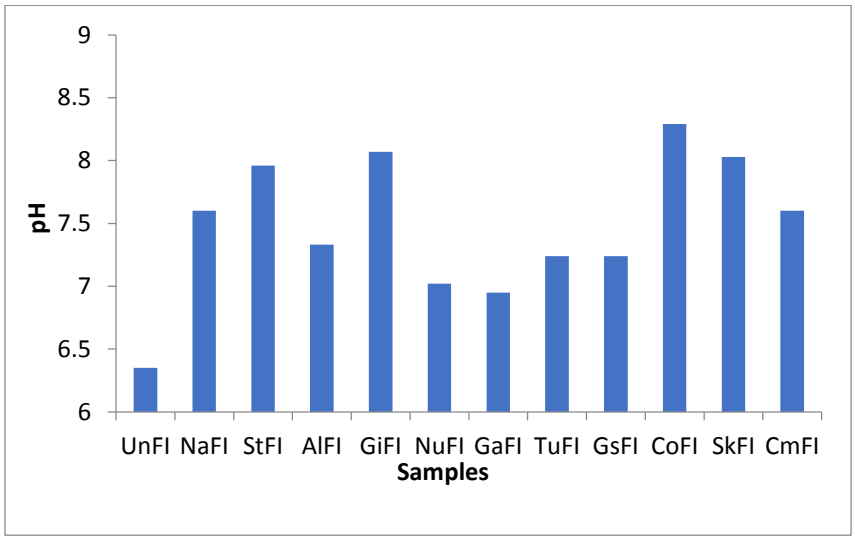

Fig 2: pH of unfermented 'iru' and spices fortified fermented 'iru'

Key: UnFI = Unfermented iru, NaFI = Naturally fermented iru, StFI = Starter culture fermented iru, AiFI =Alligator fermented iru, GiFI = Ginger fermented iru, NuFI = Nutmeg fermented iru, GaFI = Galic fermented iru, TuFI = Tumeric fermented iru, GsFI = Grain of selime fermented iru, CoFI = Cocoplum fermented iru, SkFI = Skinplum fermented iru, CmFI = Commercially fermented iru.

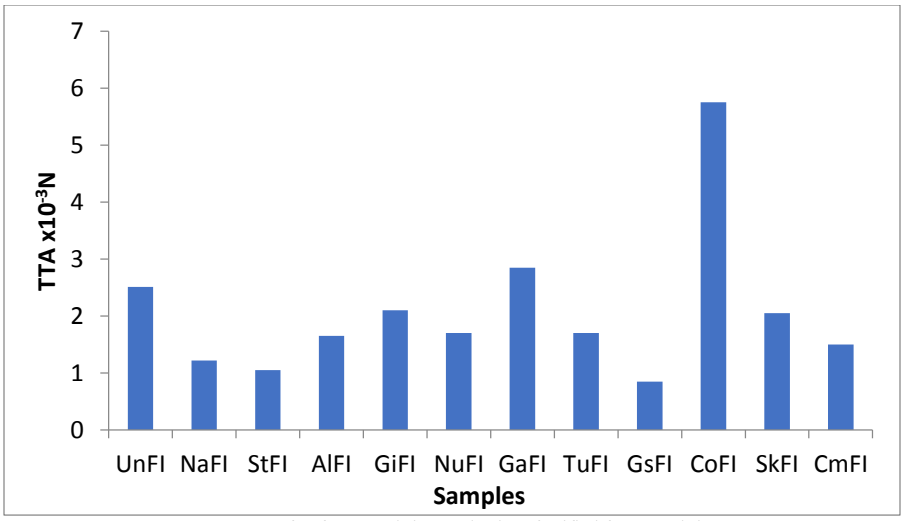

Fig 3: TTA of unfermented 'iru' and spices fortified fermented 'iru'

Key: UnFI = Unfermented iru, NaFI = Naturally fermented iru, StFI = Starter culture fermented iru, AiFI =Alligator fermented iru, GiFI = Ginger fermented iru, NuFI = Nutmeg fermented iru, GaFI = Galic fermented iru, TuFI = Tumeric fermented iru, GsFI = Grain of selime fermented iru, CoFI = Cocoplum fermented iru, SkFI = Skinplum fermented iru, CmFI = Commercially fermented iru.

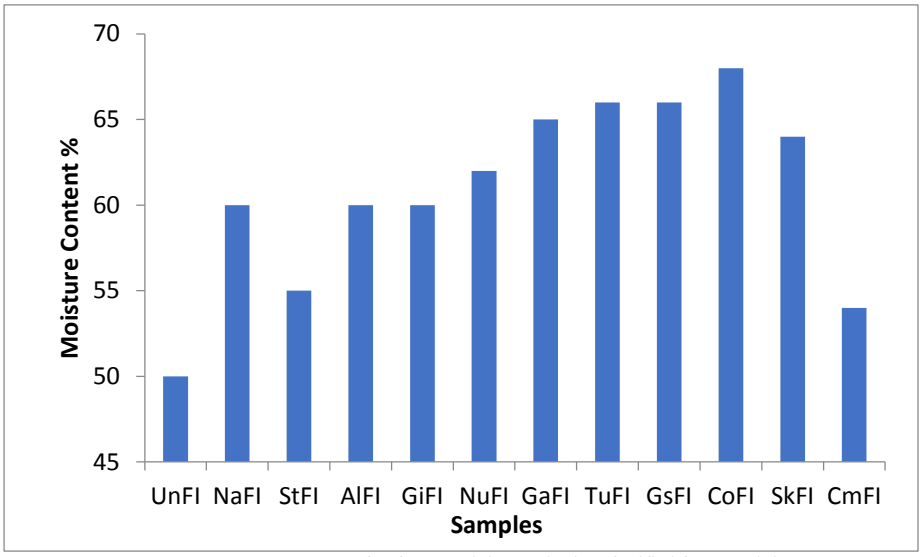

Fig 4: Moisture content (%) of unfermented 'iru' and spices fortified fermented 'iru'

Key: UnFI = Unfermented iru, NaFI = Naturally fermented iru, StFI = Starter culture fermented iru, AiFI =Alligator fermented iru, GiFI = Ginger fermented iru, NuFI = Nutmeg fermented iru, GaFI = Galic fermented iru, TuFI = Tumeric fermented iru, GsFI = Grain of selime fermented iru, CoFI = Cocoplum fermented iru, SkFI = Skinplum fermented iru, CmFI = Commercially fermented iru.

Table 1: Proximate composition (%) of unfermented 'iru' and spices fortified fermented 'iru'

Samples	Protein	Ash	Crude fibre	Fat	Carbohydrate
UnFI	20.19^{k} ±0.80	4.30^{j} ±0.02	8.23^{a}±0.30	13.7^{i}±0.80	36.81^{a}±1.15
NaFI	39.93^{c}±0.02	3.79^{g} ±0.01	5.46^{c}±0.06	19.75^{a}±0.06	25.77^{i}±0.78
StFI	41.22^{b}± 0.03	5.04^{c} ±0.01	3.50^{d} ±0.14	15.71^{g} ±0.01	24.55^{j} ±0.04
AlFI	35.79^{g} ±0.02	2.86^{i} ±0.00	2.78^{h} ±0.00	16.05^{f} ±0.00	34.27^{d} ±0.00
GiFI	41.85^{a} ±0.07	4.87^{d} ±0.01	3.39^{e} ±0.01	19.75^{a} ±0.03	23.84^{k} ±0.07
NuFI	39.93^{c} ±0.04	2.95^{h} ±0.00	5.46^{c} ±0.41	19.70^{b} ±0.00	25.77^{i} ±0.00
GaFI	37.89^{e} ±0.02	5.06^{b} ±0.00	3.41^{f} ±0.01	14.05^{i} ±0.05	28.27^{h} ±0.03
TuFI	32.70^{i} ±0.02	3.79^{g} ±0.06	3.01^{g} ±0.00	17.83^{d} ±0.02	35.78^{b} ±0.26
GsFI	33.75^{h} ±0.07	6.07^{a} ±0.00	1.48^{i} ±0.00	18.51^{c} ±0.00	33.64^{e} ±0.57
CoFI	39.81^{d} ±0.02	3.99^{f} ±0.08	2.42^{h} ±0.00	16.94^{e} ±0.00	31.80^{f} ±0.71
SkFI	36.39^{f} ±0.02	4.73^{e} ±0.00	1.17^{j} ±0.01	15.13^{h} ±0.05	35.24^{c} ±0.00
CmFI	30.03^{j} ±0.01	3.99^{f}±0.43	6.03^{b}±0.45	15.13^{h}±0.40	29.48^{g}±1.01

Key: UnFI = Unfermented iru, NaFI = Naturally fermented iru, StFI = Starter culture fermented iru, AiFI =Alligator fermented iru, GiFI = Ginger fermented iru, NuFI = Nutmeg fermented iru, GaFI = Galic fermented iru, TuFI = Tumeric fermented iru, GsFI = Grain of selime fermented iru, CoFI = Cocoplum fermented iru, SkFI = Skinplum fermented iru, CmFI = Commercially fermented iru.

The pleasant flavour and aroma obtained from local fermented condiments can be attributed to the proteolytic action of the microorganisms on the seed. In local fermented foods, microorganisms play major roles in the preparation and preservation of the food products (Achi, 2005; Ifesan et al., 2019). Fermented foods possess several nutritional benefits to the consumer and as well protect the food against food spoilage organisms. It is immediately apparent that the fermentation of *Parkia biglobosa* seeds is an alkaline based fermentation, as confirmed by Ojewumi et al (2021), with the pH ranging from neutrality to above 7.0. pH of 'iru' samples in this study were found to be within the ranges reported for legumes in the literatures (Ifesan et al., 2019). Naturally fermented 'iru' and commercially fermented 'iru' had same pH values. This is not surprising because the commercially fermented *Parkia biglobosa* seeds undergo spontaneous fermentation naturally. The alkaline fermentation of *Parkia biglobosa* is attributed to ammonia production, whose production is due to the proteolytic activity by microorganisms taking place during fermentation, thereby raising the pH of the condiment and giving it a strong ammonia odour (Ineabuchi et al., 2014; Ifesan et al., 2019). *B. subtilis* possesses ability to hydrolyze protein to give proteases, amino acid and ammonia. This acts as sources of energy and carbon for growth. Dissolved ammonia results to an upshoot in the level of alkalinity in media (Ojewumi et al., 2021). Results of the proximate analysis revealed that the unfermented 'iru' had the least moisture content, this could be expected because the seeds are in their natural state with no added water. The high moisture content of the fermented condiments could be as a result water added during pretreatment such as cooking. It may also be due to the activity of the fermenting organisms on the substrate, this result is in agreement with the result of Omafuvbe et al. (2004) while carrying out similar research on African Locust bean and melon; as well as Ibrahim et al.'s (2020a) findings on similar work on 'iru' produced from locust beans and soya beans. is an indication of early spoilage. Considering the higher moisture content of all the fermented samples compared to unfermented and commercially produced samples, there is possibility of the spices to aid absorption of moisture. Protein content of unfermented 'iru' was recorded as the least. This clearly showed that fermentation positively impacted the protein content of all the fermented samples. The significant increase in protein content of all fermented condiments could be due to proteolytic activities of the fermenting organisms during fermentation (Enujiugha, 2003; Ifesan *et al.,* 2019). Protein of all the samples was higher than what was reported by Ibrahim et al (2020a) in *Bacillus subtilis* fermented 'iru'. The high protein content in these fermented spiced condiments could be a valuable and cheap source of dietary protein where animal proteins are presently highly unaffordable to majority of the populace (Ibrahim et al 2020a). Ibrahim et al. (2020b) also recorded similar higher protein value in 'iru' fermented with *B. subtilis* A_2 when compared to the naturally fermented iru.

Decrease in the carbohydrate content of all fermented iru samples as compared to the unfermented locust bean seeds may be as a result of the ability of the microorganisms to utilize it as their major carbon source during fermentation, and this is in agreement with the findings of (Omafuvbe et al., 2004; Jonathan et al., 2011; Amao et al., 2013; Ibrahim et al., 2020b) who reported decrease in the percentage carbohydrate contents of different fermented legumes after fermentation for production of various condiments. The reduction in carbohydrate content may as well be due to the hydrolytic effect of microbial amylase converting carbohydrate into sugars (Ibrahim et al., 2020b).

The lowered ash content of the commercially produced, naturally fermented iru as well as iru fermented with alligator pepper, nutmeg, turmeric, and cocoplum as compared to unfermented seeds may be as a result leaching out of the soluble inorganic minerals into the processing water during the boiling of the bean (Ibrahim et al. 2020a). However, higher ash contents recorded in starter fermented iru and iru fermented with ginger, garlic, grain of selim and skinplum could be associated with decreased antinutrients in the seeds, which results to bioavailability of minerals in the media, hence, increased ash content (Ndidi et al., 2014; Samtiya et al., 2020). This could also be due to the release of additional minerals from the spices.

The significantly lowered crude fibre content of all the fermented locust bean seeds could be as a result of release of cellulolytic enzymes by the fermenting microbes (Farinde et al., 2011). All fermented samples had higher fat contents. The increase in fat content of the fermented iru samples may be attributed to increased activities of lipolytic enzymes, which hydrolyze fat to glycerol and fatty acid (Ibrahim *et al.,* 2020a). High fat content in food could result to rancidity, hence, quick spoilage of the fermented condiments (Othón-Díaz *et al.,* 2023).

Conclusion: The study showed that fermentation of *Parkia biglobosa* seeds with the use of *Bacillus subtilis* as starter culture will produce a nutritionally rich condiment. Findings from this research has also

confirmed that locust bean seeds can be fermented alongside spices to further improve the nutritional composition and nutraceutical properties of the condiment.

REFERENCES

Achi, OK (2005). The Potentials for upgrading traditional fermented foods through biotechnology *Afr. J. Biotech.* 4(5):375- 380.

Adelekan, AO; Uzochukwu, SVA; Esiobu, N (2017). Bacterial diversity study on fermentation of *Parkia biglobosa* using 16s rRNA gene analysis. *Int. J. Appl. Microbiol. Biotechnol*. 5: 95-102. DOI: https://doi.org/10.33500/ijambr.2017.05.012

Amao, JA; Abel, OO; Agboola, JO (2013). Proximate analysis and sensory evaluations of iru produced by Staphylococcus sp. and Bacillus sp. separately. *IOSR J. Environ. Sci., Toxicol. Food Technol.* 6(2): 26-30.

AOAC, (2000). Official Methods of Analysis, Association of official Analytical Chemists, Washington, DC, USA, 17th edition

Das, G; Sharma, A; Sarkar, PK (2022). Conventional and emerging processing techniques for the post-harvest reduction of antinutrients in edible legumes. *Appl. Food Res*. 2(1): 1-12. DOI: https://doi.org/10.1016/j.afres.2022.100112

Enujiugha, VN (2003). Nutrient changes during the fermentation of African oil bean (Pentaclethra macrophylla Benth) seed. *Pakist. J. Nutr*. 2: 320-323. DOI: doi.org/10.3923/pjn.2003.320.323

Farinde, EO; Adeniran, HA; Abiose, HE (2011). Proximate Composition, Mineral Content and Sensory Assessment of an Iru Analogue Produced from Fermented Lima Bean (Phaseolus Lunatus). *Ife J. Technol*. 20(2): 1-6

Ibrahim, TA; Agbaje, RB; Osadare, OP; Falusi, V; Gbadamosi, OA (2020a). Proximate and Antinutrient Composition of Iru Produced from African Locust Beans and Soybeans Using Bacillus subtilis A2 as Starter Culture. *Int. J. Res. Innov. Appl. Sci*. 5(1): 174- 176

Ibrahim, TA; Omosuli, SV; Oloye, DA; Adewale, CA; Osanipin, OH (2020 b). Comparative Chemical Composition of Iru Fermented spontaneously and with Bacillus subtilis A2 as Starter Culture. *Glob. Sci. J.* 8(1): 253- 262

Ifesan, BOT; Adetogo, TT; Ifesan, BT (2019). Production and Quality Assessment of Local Condiment 'Ogiri' from Watermelon Seed (*Citrullus lanatus*) and Melon (*Citrullus vulgaris*). *Adv. Food Process. Technol*. 2(1): 1-6. DOI: https://doi.org/10.29011/2639-3387.1000022

Ineabuchi, JC; Olawuni, IA; Iheagwara, MC; Ojukwu, M; Ofoedu, CE (2014). Microbiological Evaluation of 'Iru' and 'Ogiri-Isi' used as Food Condiments. *IOSR J. Environ. Sci., Toxicol. Food Technol.* 8(1): 45-50

Jonathan, SG; Fadahunsi, IF; Garuba, EO (2011). Fermentation studies during the production of iru from Bambara nut (Voanzeia subterranean I. Thours), and indigenous condiment from south-western Nigeria. *Elec. J. Env. Agricult. Food Chem.* 10(1), 1829-1836

Mokoena, MP; Mutanda, T; Olaniran. AO (2016). Perspectives on the probiotic potential of lactic acid bacteria from African traditional fermented foods and beverages. *Food Nutr. Res*. 2016, 60: 29630. DOI: http://dx.doi.org/10.3402/fnr.v60.29630

Ndidi, US; Ndidi, CU; Olagunju, A; Muhammad, A; Billy, FG; Okpe, O (2014). Proximate, Antinutrients and Mineral Composition of Raw and Processed (Boiled and Roasted) *Sphenostylis stenocarpa* Seeds from Southern Kaduna, Northwest Nigeria. *Int. Sch. Res. Notices.* 14:9 DOI: https://doi.org/10.1155/2014/280837

Odebunmi, EO; Oluwaniyi, OO; Awolola, GV; Adediji, OD (2009). Proximate and nutritional composition of kola nut (*Colanitida*), bitter cola (*Garcinia cola*) and alligator pepper (*Afromomum melegueta*). *Afr. J. Biotechnol*. 8(2): 308-310. DOI: https://doi.org/10.4314/ajb.v8i2.59797

Ojewumi, ME; Omoleye, AJ; Ajayi, AA (2016). The Effect of Different Starter Cultures on the Protein Content in Fermented African Locust Bean (*Parkia Biglobosa*) Seeds. *Int. J. Eng. Res. Technol.* 5(4): 249-255

Ojewumi, ME; Omoleye, JA; Ajayi, AA; Ekanem, GP (2021). Fermentation rate monitoring in the production of African protein based condiments. *IOP Conference Series: Earth Environ. Sci.* 655(1):012012.DOI: https://doi.org/10.1088/1755-1315/655/1/012012

Olutiola, PO; Famurewa, O; Sonntag, HG (1991). An Introduction to General Microbiology: A Practical Approach. Hygiene-Institut Der Universitat Heidelberg, Germany. p. 257

Omafuvbe, BO; Olumuyiwa, S; Falade, BA; Osuntogun; Steve, RA; Adewusi. (2004). Chemical and biochemical changes in African locust beans (*Parkia biglobosa*) and melon (*Ctrullus vulgaris*) seeds during fermentation to condiments, *Pak. J. Nutr*. 3(3): 140-145. DOI: https://doi.org/10.3923/pjn.2004.140.145

Omodara, TR; Aderibigbe, EY (2014). Effects of starter culture and different components of 'kuuru' on the nutritional quality of fermented *Parkia biglobosa*. *Int. J. Appl. Microbiol. Biotechnol. Res*. 2: 73-78. DOI: https://doi.org/10.33500/ijambr.2014.02.008

Othón-Díaz, ED; Fimbres-García, JO; Flores-Sauceda, M; Silva-Espinoza, BA; López-Martínez, LX; Bernal-Mercado, AT; Ayala-Zavala, JF (2023). Antioxidants in Oak (*Quercus* sp.): Potential Application to Reduce Oxidative Rancidity in Foods. *Antioxidants*. 12(4): 861. DOI: https://doi.org/10.3390/antiox12040861

Sachan AK; Kumar, S; Kumari, K; Singh (2018). Medicinal uses of spices used in our traditional culture: Worldwide. *J. Med. Plants Stud*. 6(3): 116-122

Saleh, MSM; Jalil, J; Zainalabidin, S; Asmadi, AY; Mustafa, NH; Kamisah, Y (2021). Genus *Parkia*: Phytochemical, Medicinal Uses, and Pharmacological Properties. *Int. J. Mol. Sci*. 22(2):618. DOI: https://doi.org/10.3390/ijms22020618

Samtiya, M; Aluko, RE; Dhewa, T (2020). Plant food anti-nutritional factors and their reduction strategies: an overview. *Food Prod. Process. Nutr.* **2**, 6. DOI: https://doi.org/doi.org/10.1186/s43014-020-0020-5

Voidarou, C; Antoniadou, M; Rozos, G; Tzora, A; Skoufos, I; Varzakas, T; Lagiou, A; Bezirtzoglou, E (2020). Fermentative Foods: Microbiology, Biochemistry, Potential Human Health Benefits and Public Health Issues. *Foods*, 10(1):69. 1-27. https://doi.org/doi:10.3390/foods10010069