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Suitability of bacterial fermentation and foil packaging of condiment from African mesquite (*Prosopis africana*) seeds for nutritional retention and commercialization

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The process for bacterial fermentation and foil packaging of condiment ('Okpeye') from African mesquite seeds was described and its nutritional enrichment and packaging improvement for commercialization were verified. The seeds (1 Kg) were cleaned, washed and boiled in autoclave at 121°C for 2 h, cooled, drained, de-hulled and divided into two portions of 300 g each. One portion was placed in sterile paw-paw leaves (BL), inoculated and tightly wrapped with the leaves to ferment for 4 days. The remaining portion was placed in aluminium foil, inoculated and wrapped tightly with the foil (BF). Seeds processed by the traditional method served as control (TL and TF). The condiments were subjected to selected nutritional composition and sensory analyses. Moisture content was significantly ($p<0.05$) higher for TL product than TF and could indicate that higher moisture was absorbed from the leaves during fermentation of de-hulled seeds than from the foil. Negligible differences were found in the ash and carbohydrate contents of the samples. Crude protein contents were higher for bacterial fermented products (26.70-27.53%) than traditional products (18.07-18.67%). The BL condiment was preferred to others and was overall most acceptable. The study advocates bacterial fermentation for the production of the condiment, its cubing and packaging in the foil; for nutrient retention and income generation.

Key words: Mesquite seeds, condiment, *Bacillus* species, fermentation, foil packaging.

INTRODUCTION

Fermented African mesquite ('Okpeye') is a condiment used during food preparations for flavor enhancement, micronutrient and protein enrichment in Nigeria and beyond (Keay, 1989). Consequently, this condiment is utilized similarly as 'Dawadawa/iru' from African locust bean, soybean, among other legumes and 'Ogiri' from castor oil or fluted pumpkin seeds, while preparing foods (Achi, 2005). However, all the condiments have been processed indigenously using traditional methods that might involve uncontrolled solid substrate fermentation. This method as reported by Arogba et al. (1995) and Olasupo et al. (2016) could lead to excessive hydrolysis

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of the protein and carbohydrate portions. Hence, the condiments have not achieved high commercial status due to very poor shelf-life, unsuitable packaging and objectionable odor as indicated by the findings of Eka (1980). African mesquite seeds were found to be inedible in the raw unfermented state due to high concentrations of anti-nutritional factors that could be very dangerous to human health if consumed without processing (Achi, 2005).

Hence, de-hulled seeds undergo alkaline-fermentation where oligosaccharides and proteins, among others, are broken down to other smaller molecules before consumption as fermented condiment. De-hulled seeds were fermented within 4-5 days wrapped up in pawpaw or any other non-toxic leaves in the traditionally setting (Afolabi et al., 2018). Findings of Oguntoyinbo et al. (2007) and Afolabi et al. (2018) on various species of *Bacteria* isolated from 'okpehe', a traditional condiment from African mesquite seeds in Nigeria, indicated that microorganisms actively involved in the fermentation of the seeds were Bacillus species specifically *Bacillus subtilis*, *Baccillus pumilus*, *Baccillus licheniformis* and *Baccillus megaterium*. The species were also noted to be lower acid producers than lactic acid bacteria (Parkouda et al., 2009). This could be one of the reasons why the pH of the process rises from neutral to alkaline levels (Ibrahim et al., 2018; Afolabi et al., 2018) in most cases during production. Alkaline-fermentation by these microorganisms gave rise to enhanced digestibility, nutritional contents, aroma and flavor of the raw seeds, among other benefits (Balogun et al., 2014; Gutierrez et al., 2016).

Due to preservation and convenience, the availability of mesquite condiments in powdered and cubed forms would make packaging easier and improve its acceptability. It can also assist in easy incorporation into soups and stews, among others, without further grinding. Oguntoyinbo (2014) highlighted in a review on safety challenges facing traditional foods of West Africa that lack of attention to type of packaging material, unhygienic and low standard would expose the fermented foods to various contaminations. In research studies of Balogun et al. (2014) and Gberikon et al. (2015), the powdered condiment was produced by using inoculants made up of two species of *Bacillus* organisms (*Bacillus subtilis* and *Bacillus licheniformis*) but the products were not cubed. Also, information on cubing and proper packaging for shelf-life stability was limited. Observation in the local environment showed that the powdered forms were now packaged in polyethylene materials and sold by small scale business entrepreneurs in certain markets.

The most current method of storing and marketing mesquite condiment by the traditional producers is molding the partially sun-dried product (50-70% moisture loss) between palms before being sold. The products were then allowed to complete drying as exposed on the shelves during sales. In the study, aluminum foil was used to package the condiments after cubing. This was because aluminum foil has been highly regarded as important packaging material in laminates with broad application in food packaging (Manuela and Felix, 2007). The obvious use of aluminum in food preservation was its potential to provide a complete barrier against light, oxygen, moisture, and bacteria including reduction in the losses of volatile aroma. Also, aluminum foil is inert but can react with few compounds such as sodium hydroxide in the presence of moisture. Hence, the objective of the study was to evaluate suitability of bacterial fermentation and foil packaging of condiment from African mesquite (*Prosopis africana*) seeds for nutritional retention and generation of income.

MATERIALS AND METHODS

For the study 2 kg of African mesquite seeds were bought from 'Ogige' market in Nsukka metropolis of the University town. Pure culture used for the fermentation, *Bacteria inoculums* (NRRL B-571), was procured from the United States Department of Agriculture (USDA) Gene Bank. A hot air oven (LAB AIDS, Model number-1201; made in India) in the Food Science and Technology laboratory was also used, among others.

Fabrication of the moulds for cubing

Coated aluminium sheets were measured and cut into flat trays and then in cubic shapes. The fabrication was done in a welder's workshop at the mechanic village in Nsukka metropolis. The moulds were placed inside trays after thorough cleaning and sterilization and used to form shapes for the condiment. The cubed condiments were manually packaged in aluminium foil (Figure 2).

Preparation of sample and production of condiment using traditional method

African mesquite condiment was produced traditionally using the method described by Ugwuarua (2010). Raw mesquite seeds (1000 g) were cleaned to remove extraneous materials. The seeds were washed in clean water, drained and boiled for 12 h. The boiled seeds were de-hulled and cotyledons boiled in enough quantity of water for one hour and drained. The boiled cotyledons were allowed to cool, divided into two portions of 300 g each, wrapped with sterile paw-paw leaves (TL) and aluminium foil (TF) and left to ferment inside food processing room (28 ± 2°C) for four days with the control samples. The fermented mash was ground to form a paste, moulded into shapes and dried partially to a moisture content of 40%) in a hot air oven at 50°C for the formation of the cubes.

Preparation of sample and production of mesquite condiment using bacterial fermentation

Mesquite seeds (1000 g) for bacterial fermentation were cleaned, washed and boiled in Autoclave at 121°C for 2 h (Gberikon et al., 2015), cooled, de-hulled (Figure 1(i)), and boiled for half-hour for more sterilization. They were divided into two portions of 300 g each (Ogbadu and Okagbue, 1988) and placed inside sterilized (Balogun et al., 2014) stainless steel bowls, lined with sterilized aluminum foil to cool to 30°C without exposing to the air before inoculation took place. The second portion was also quickly

(i). De-hulled seeds

(ii) Sample fermented in Aluminum foil

(iii). Sample fermented in paw-paw leaves

Figure 1 (i-iii): Pictorial presentation of de-hulled and fermented mesquite seeds.

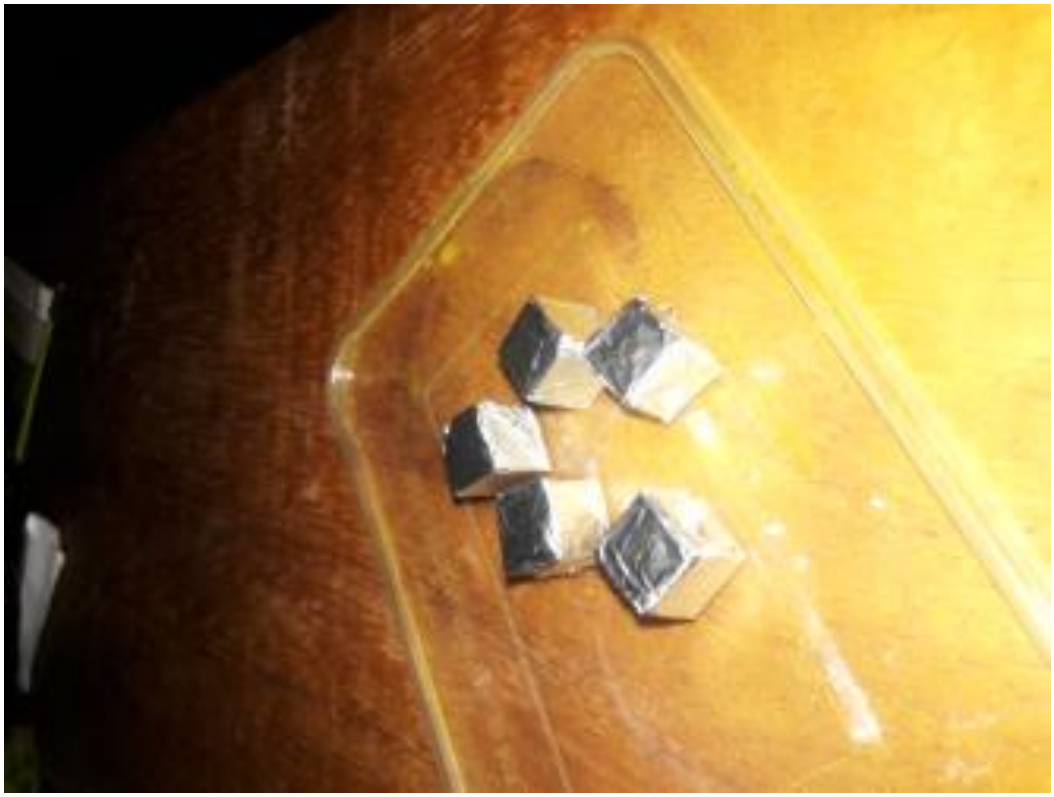


Figure 2. Pictorial presentation of the cubed packaged condiment.

wrapped up with sterilized pawpaw leaves after inoculation. *Bacillus* inoculums for controlled fermentation of *P. africana* seeds was prepared according to the method described in Gberikon et al. (2015). The inoculums (mixed strains of *B. subtilis* and *Bacillus pumilus*) used contained 2.7×10^7 cells/ml. The cell population was calibrated using McFarland standards (No. 7) which was prepared by adding 0.7 ml of 1% anhydrous barium chloride to 9.3 ml of 1% sulphuric acid.

Inoculation was done based on 5.0% (that is, 5% of the weight of de-hulled seeds) of fermenting materials and inoculum was kept for 24 h for stabilization of the organisms before use. Hence, 15 ml each was inoculated into the two portions and wrapped separately with sterile paw-paw leaves and aluminum foil for fermentation to take place (BL and BF) at the laboratory room temperature (28

±2.0°C). Figure 1(ii-iii) shows the samples obtained after the fermentation of de-hulled seeds (Figure 1 (i)).

Analytical methods

Proximate analysis of the condiments

Prepared samples were analyzed for proximate composition (moisture, ash, crude fat, crude protein, and total carbohydrate) according to the Association of Official Analytical Chemists-AOAC (2010) method.

Selected minerals and pro-vitamin A of the samples

Analysis of selected minerals (calcium and iron) of the condiments was done using the method of Kirk and Sawyer (1991), while pro-vitamin A was carried out by the method described in Anonymous (2019) and Anna and Stefano (1992). In the procedure, samples were first saponified using an alcoholic solution of potassium hydroxide in the presence of pyrogallol to absorb any molecular oxygen that can cause oxidation. The un-saponified matter containing vitamin A was extracted using a mixture of diethyl ether and petroleum spirit. The extract was evaporated under nitrogen gas and the residue dissolved in methanol. The extract was chromatographed using a reverse-phase ODS (Octadecylsilica) column with the mobile phase consisting of 95% acetonitrile with 5% water. The separated pro-vitamin A was quantified using a UV absorbance detector at 328 nm.

Microbial analysis of the samples

Total viable count

The total viable counts for condiments were carried out using the method described by Prescott et al. (2005). One gram of the sample and 9 ml of ringer solution were used to make serial dilutions up to 10^{-3}. The diluted sample was pipetted into a marked Petri dish. 15 ml of prepared nutrient agar solution was added; the solution was swirled to mix and incubated at the temperature of 37°C (Hyvarinen et al., 1991) for 24 h. After incubation, the number of colonies was counted and represented as colony-forming unit per gram (CFU/g).

Mold count

Mold count determination for condiments was done according to the method described by Prescott et al. (2005). The media used was Sabouraud dextrose agar. Then, 15 ml of Sabouraud dextrose agar solution was added to one gram of sample in the Petri dish. It was thoroughly mixed and allowed to set before incubating at a temperature of 37°C for 48 h. After incubation, the number of colonies was counted and represented as colony-forming unit per gram.

Sensory evaluation of the samples

Twenty untrained panellists (within 18-35 years in age) randomly selected from the Department of Food Science and Technology, University of Nigeria, Nsukka, were used to evaluate the condiment samples. The samples were evaluated for appearance, color, odor, finger feel, and overall acceptability. The extent of differences among samples for each sensory attribute was measured using a 9-point Hedonic scale where 9 represents ‘extremely like’ and 1 represents ‘extremely dislike’ (Ihekoronye and Ngoddy, 1985).

Experimental design and data analysis

The experimental design was based on a completely randomized design. The data from the analyses were subjected to a one-way analysis of variance using the Statistical Product for Service Solution (SPSS) version 20.0. Means were separated by Duncan’s new multiple range test and the level of significance was accepted at $p<0.05$ (Obi, 2001).

RESULTS AND DISCUSSION

Proximate composition of fermented mesquite seeds

Proximate composition of the fermented condiment is presented in Figure 3 while the cubed condiment packaged in aluminum foil is shown in Figure 2. Moisture content ranged from 44.67-58.0%. Products from de-hulled seeds wrapped with leaves (TL and BL) during fermentation had higher moisture content than those with aluminum foil (TF and BF). This could be due to the presence of high moisture composition of *C. papaya* leaves shown to be 77.5 mg/100 g of the leaves by Farhan et al. (2014). Hence, fermented products would have absorbed some moisture from the leaves (Paul et al., 2018). High moisture content could introduce microbial spoilages in the food condiments as pointed out by Iwe et al. (2016), leading to poor shelf-life. Hence, further drying should be done to properly preserve the condiments as reported in Balogun et al. (2014). Moreover, findings of Nath and Dutta (2016) on phytochemical and proximate analyses of *C. papaya* leaves indicated that the leaves were rich in protein and ash including vitamins; Pro-vitamin A, C and E. Minerals such as calcium, zinc, magnesium, potassium, manganese, and iron were also found in high amounts from paw-paw leaf extracts.

Significant ($p>0.05$) differences did not exist in the values for ash (3.38-3.63%) and carbohydrate contents (9.41-9.96%) of the samples and could imply that these parameters were similarly affected during fermentation of the traditional and inoculated de-hulled samples. However, for crude fat content (Figure 3), samples TF and BF (Samples wrapped in foil during processing) had higher values than TL and BL. This could be due to the breaking down of the fats in the leaves by hydrolytic rancidity from lipase activity since paw-paw leaves contained high moisture. The flavour of the products might be affected including the shelf-life on the long run. Studies of Ibrahim et al. (2018) on effect of fermenting organisms on proximate composition of dawadawa botso suggested that the type of microorganisms present can affect nutritional values of the final product. Pawpaw leaves were also indicated to contain enzymes such as papain, chymopapain, amylase, and protease enzymes,

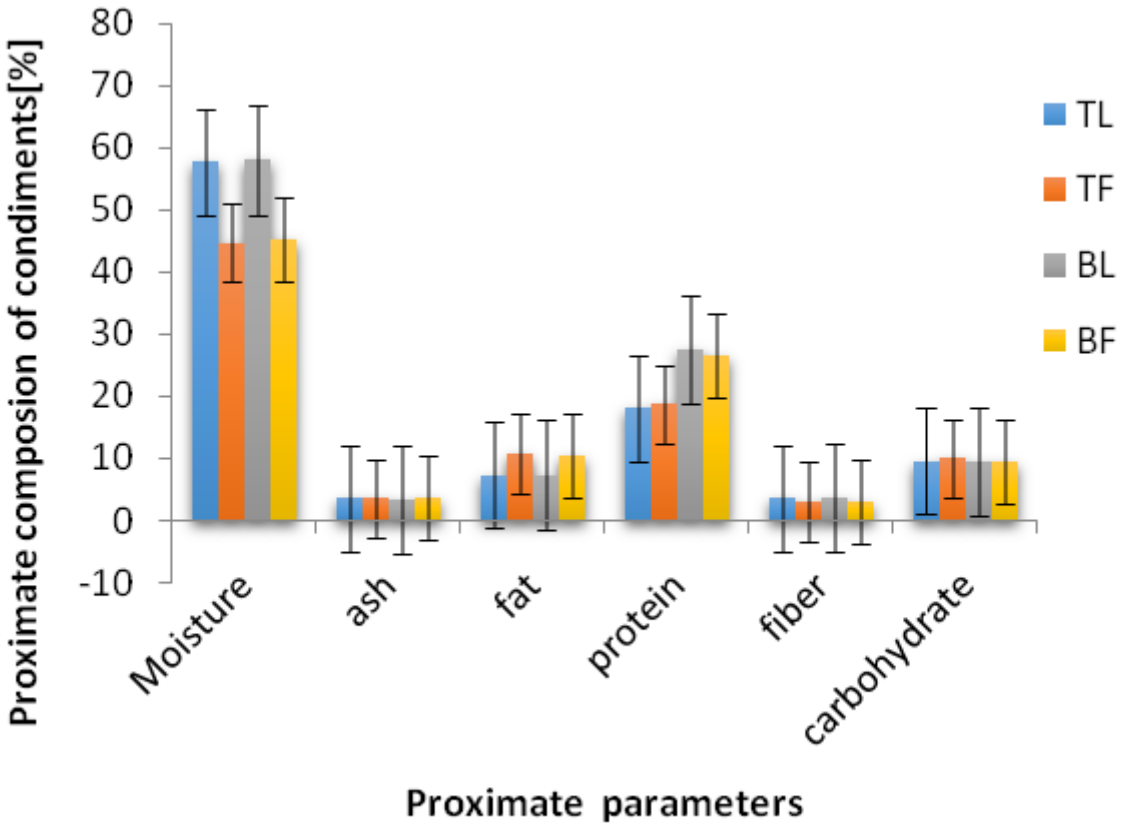


Figure 3. Proximate composition of the condiment wrapped in different materials during fermentation period. TL-de-hulled seeds fermented traditionally wrapped in paw-paw leaves; TF- de-hulled seeds fermented traditionally, wrapped in aluminum foil; BL-de-hulled seeds fermented using inoculant, wrapped in paw-paw leaves; BF-de-hulled seeds fermented using inoculant, wrapped in aluminum foil.

that moderately hydrolyze carbohydrates and proteins, respectively (Saeed et al., 2014). Protein content of the products ranged from 18.67-27.53% and was significantly ($p< 0.05$) higher in the condiments BL and BF (Bacteria inoculated samples) compared to TL and TF. The increase in crude protein content agreed with values obtained by Gernah et al. (2005) and in the studies of Campbell-Platt (1980) on the production of 'Dawadawa'. The crude protein increased from 24.8-33.5% and 30.0-38.50%, respectively. The increase in the protein contents might be due to breaking down of oligosaccharides and proteins, among other molecules, in the seedy legumes into non-toxic substrates, amino acids and ammonia, etc, mainly carried out by the *Bacillus* species (Maji and Adegoke, 2019). These species could have higher population in the inoculated de-hulled seeds than in the control. This was why the pH during fermentation of the seeds increased from neutral to alkaline levels as discovered by Ibrahim et al. (2018) and Maji and Adegoke (2019), among others. Further, TL and BL condiments (samples wrapped in leaves during fermentation) had higher values in crude fiber than TF and BF (samples wrapped in the foil). This might be due to degradation of fiber in the seeds during fermentation by *B. subtilis*, to produce other substances (Amoa-Awua and Jakobsen, 2008). Values recorded showed that wrapping material for de-hulled seeds during processing might influence fibre contents and other proximate parameters of the final product which may also depend on the fermenting organisms present.

Selected micronutrients of the condiments

Selected micronutrient composition (Calcium, Iron and Pro-Vitamin A) of all processed samples are presented in Figure 4. The TL condiment had the highest value in iron composition (28.20 mg/100 g) among all the samples. Nda-Umar et al. (2008) also detected iron in amounts such as 79.38 mg/100 g) in fermented *P. africana* condiment locally called 'Okpehe', while 15.50 ± 0.4 mg/100 g, of iron, were obtained by Aremu et al. (2006) in *P. africana* flour. Experimental results underscored why in the traditional setting *P. africana* tree is called an iron tree. Nevertheless, sample TF (25.77 mg/100 g) had a higher value in iron content than BF

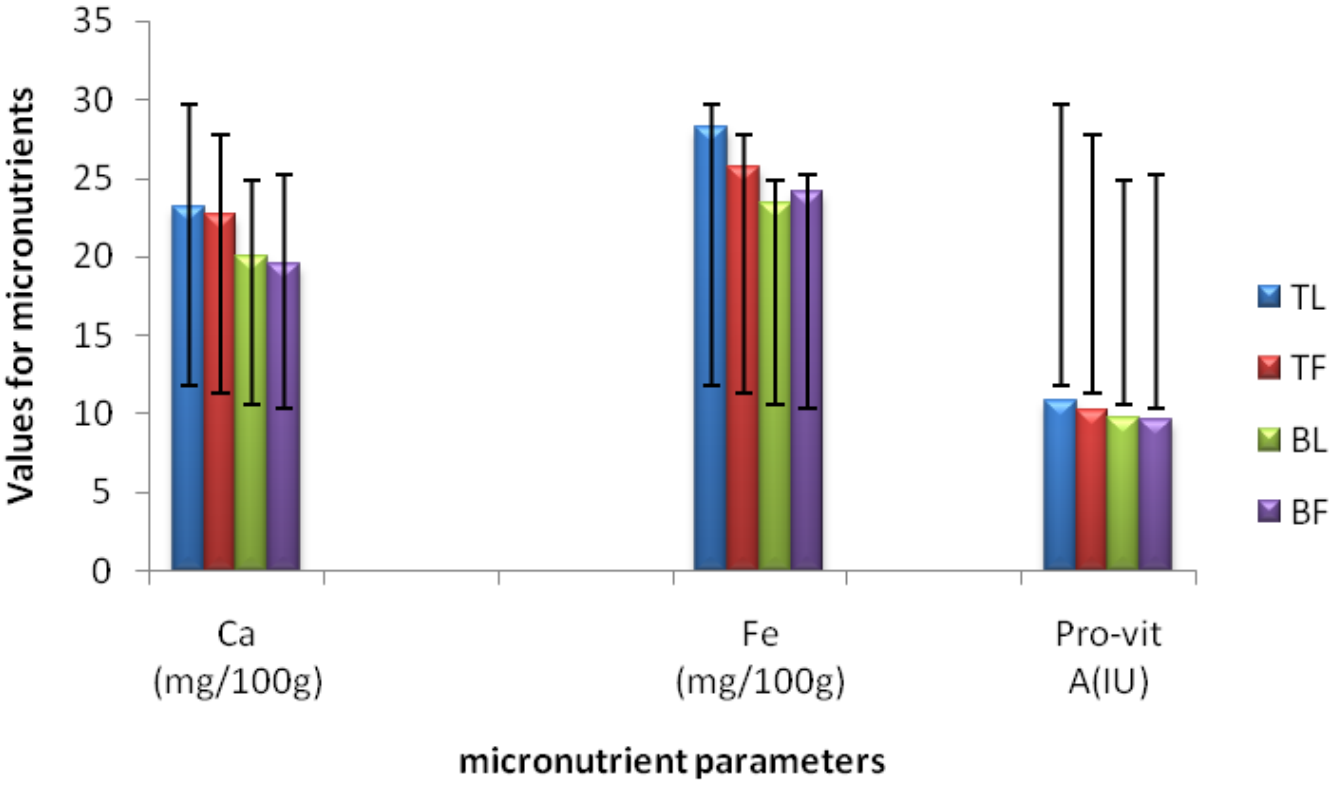


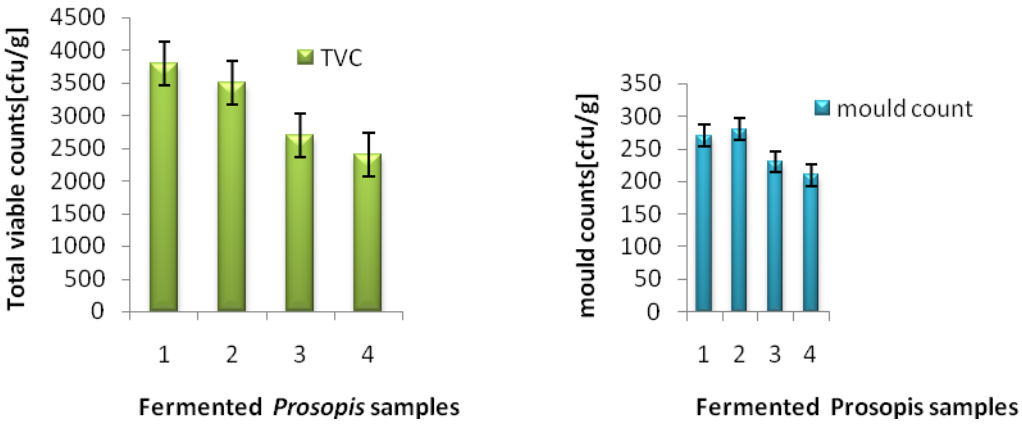
Figure 4. Selected micronutrients composition of fermented samples.TL-de-hulled seeds fermented traditionally wrapped in paw-paw leaves; TF- de-hulled seeds fermented traditionally, wrapped in aluminum foil; BL-de-hulled seeds fermented using inoculant, wrapped in paw-paw leaves; BF-de-hulled seeds fermented using inoculant, wrapped in aluminum foil.

(24.17 mg/100 g). Iron carries oxygen to the cells and is necessary for the production of energy, synthesis of collagen and the proper functioning of the immune system (Anhwange, 2008). All samples were fair sources of pro-vitamin A and regular consumption of the condiments could help in the maintenance of the human vision. Products from the traditional method of processing had higher values in pro-vitamin A contents (TL-10.83IU; TF-10.23 IU) than inoculated products (BL-9.70 IU; BF-9.60 IU).

This might be due to the heat of fermentation present in samples wrapped with aluminum foil compared to those in the leaves during the fermentation period. Pro-vitamin A was easily oxidized in the presence of high heat content (Vickie and Christian, 2008). Calcium ranged from 19.53-23.20 mg/100 g. The content was significantly ($p<0.05$) higher for samples TL and TF than BL and BF. This could be due to more consumption of calcium by *B. species* for their metabolic activities in the foil than in mixed culture fermentation (Ibrahim et al., 2018). The presence of calcium in the condiment is beneficial because it is needed for strong bone and teeth formation in humans. The general results indicated that all condiments were fair sources for the selected micronutrients and would contribute to a healthy lifestyle when consumed regularly. The study also showed that use of mixed culture fermentation of the traditional method could assist selected minerals in being more bio-available than using only *Bacillus* species contrary to what was observed by Mohite et al. (2013) in their findings.

Microbial counts of mesquite condiment

Microbial counts of mesquite condiments are shown in Figure 5. All samples had relatively low values for total viable and mould counts and found within the limit required by ICMSF (1978). However, the total viable and mould counts of samples TL and TF were significantly ($p<0.05$) higher than those of samples BL and BF. Similarly, products from de-hulled seeds packaged in aluminium foil (TF and BF) during fermentation had lower values in the counts than those of leaves (TL and BL). These scenarios were noted by Achi (2005), Tope (2013) and Afolabi et al. (2018) but concluded that it could be due to composition of the substrates in the traditional fermented condiments. This underscores the benefit of inoculation of de-hulled samples with known *Bacillus* species during processing.



(i) Total viable counts (ii) Mould counts

Figure 5 (i-ii). Total viable and mould counts of mesquite seeds after fermentation. Samples: 1-TL, 2-TF, 3-BL, 4-BF; TL-de-hulled seeds fermented traditionally wrapped in paw-paw leaves; TF- de-hulled seeds fermented traditionally, wrapped in aluminum foil; BL-de-hulled seeds fermented using inoculants, wrapped in paw-paw leaves; BF-de-hulled seeds fermented using inoculants, wrapped in aluminum foil.

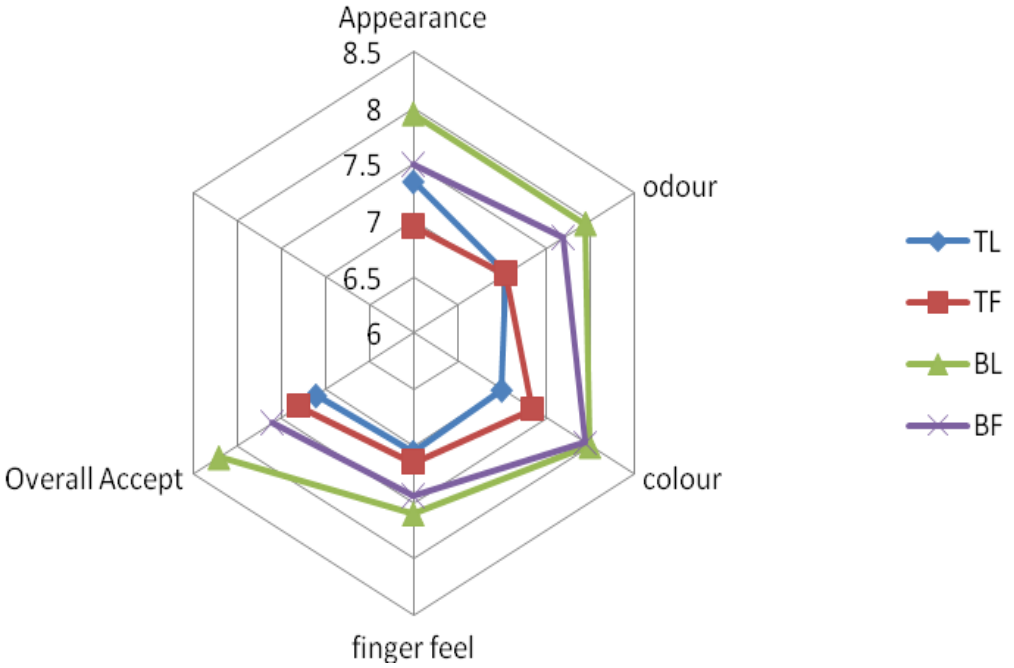


Figure 6. Spider-web plot for sensory scores of the fermented samples. TL-de-hulled seeds fermented traditionally wrapped in paw-paw leaves; TF- de-hulled seeds fermented traditionally, wrapped in aluminum foil; BL-de-hulled seeds fermented using inoculant, wrapped in paw-paw leaves; BF-de-hulled seeds fermented using inoculant, wrapped in aluminum foil.

Sensory scores of the condiment samples

Sensory scores of the condiments are shown in Figure 6. The average value of responses for all sensory attributes indicated that products from inoculated samples (BL and BF) were 'like very much' by the Panellists than traditional fermented products ('Like moderately'). However, BL condiment had slightly higher scores in all attributes than BF. The BL sample had the highest scores in odour (7.95-Like very much), appearance (7.95),

colour (8.00-Like very much), texture (finger feel-7.60; Like very much) and overall acceptability (8.2-Like very much) among others. Order of overall acceptability include; BL>BF>TF and TL.

Conclusion

The study showed that fermentation affected texture, colour, and aroma of de-hulled inoculated products from *P. africana* seeds (BL and BF) than control. Also, the fermented condiments had higher protein contents than the control products. This suggests that *Bacillus* inoculation could be encouraged in commercial production of the condiment in addition to cubing and packaging with aluminum foil for nutrient retention. The condiments were fair sources of pro-vitamin A, calcium and iron and might assist in the maintenance of a healthy lifestyle of individuals who regularly consume them through food preparations. Moreover, the inoculated fermented condiments have lower total viable and mould counts than the control products and emphasizes the benefit of using inoculation in the processing of *P. africana* seeds into fermented condiments. Therefore, study suggests *Bacillus* species' inoculation during processing, cubing and preservation with aluminum foil of the condiment for large scale commercialization to generate income and enhance household nutrition security.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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