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Effect of Fermentation on Nutrient and Anti-Nutrient Contents of Ground-Cooked Lima Bean (*Phaseolus lunatus*) Seeds using *Bacillus subtilis* and *Bacillus pumilus*

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Authors' contributions

This work was carried out in collaboration between all authors. Author KTA designed the work, carried out the research and wrote the draft of the manuscript. Authors FCA and FAA supervised the research. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aim: This study investigated the effect of single cultures of *Bacillus subtilis* and *B. pumilus* on nutrient and anti-nutrient contents of ground-cooked Lima bean seeds.
Methodology: Lima bean seeds were ground and fermented naturally as well as with single starters of *B. subtilis* and *B. pumilus* (previously isolated from naturally fermented Lima bean seeds) for nine days. Microbial analyses, pH, total titratable acidity and the temperature of the fermenting samples were carried out on daily basis, nutritional composition at two day intervals while the anti-nutrient contents were determined before and after the fermentation period.
Results: Higher microbial counts were observed in starter fermented samples than the naturally fermented samples. The pH increased throughout the fermentation period with the highest in *B. subtilis* fermented sample but was not significantly higher (P<.05) than *B. pumilus* fermented sample while the total titratable acidity which however decreased mostly in *B. subtilis* was also not significantly different from *B. pumilus* fermented sample. Temperature increased up to the fifth day in both starter samples but higher in *B. subtilis* fermented substrate but up to seventh day in naturally fermented sample. Starter culture

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fermented samples had the highest moisture, fat and ash contents at the end of the fermentation while the protein values increased in all the samples with the highest in *B. subtilis* fermented sample (25.34%) while *B. pumilus* and naturally fermented sample contained 24.32% and 22.22% respectively. Oxalate, phytic acid and cyanide were more reduced in naturally fermented sample than the starter fermented samples while the highest reductions were observed in saponin and tannin contents of *B. subtilis* fermented sample. Organoleptic parameters were rated better in the Lima bean seeds fermented with starter cultures than the naturally fermented sample.

Conclusion: This study revealed that Lima bean seeds fermented with single starter cultures of *B. subtilis* and *B. pumilus* enhanced the nutrient contents and organoleptic qualities of the products.

Keywords: Lima bean; fermentation; starter; nutrient; anti-nutrient.

1. INTRODUCTION

Legumes have been cultivated for thousands of years throughout the world. They are the next important food crop after cereals [1]. Seeds of some legumes have been reported to account for up to 80% of dietary protein as they could be the only source of protein in the diet of some group of people [2,3]. They could be cooked and eaten directly or fermented to produce condiments which serve as meat substituent for many families in developing countries. However, only few of these leguminous seeds are consumed. This has greatly contributed to dependence on cereal products which are very low in protein contents and some essential amino acids. Some of the reasons for this could be due to hard to cook syndrome and various anti-nutrient contents which they contain [2].

Inadequate protein consumption has been one of the major problems facing developing countries, particularly children both in terms of quality and quantity. This is due to the fact that cereal foods which are very low in protein contents are the major staple foods in these regions [4]. Despite the fact that some of these cereals have been upgraded through different processing methods, their protein contents have not been able to compete with those of legumes [4,5].

In spite of an urgent need to meet the nutritional requirements of the ever increasing populations, the availability of these cheap protein resources from has remained relatively unexplored [6]. They are neglected because of inadequate knowledge of processing them for both animal and human consumption. Many underutilized legumes grow well in tropical and sub-tropical countries in Africa, but the impeding need is the capacity to carry out intensive coordinated research studies in these countries in order to improve their utilization as food for improved nutritional status [7,8].

Fermentation has been employed in the production of foods and condiments from many legumes. They include *iru, ogiri, ugba, Furundu* and *tempe* from fermented African locust beans (*Parkia biglobosa*), melon seeds (*Citrullus vulgaris*), oil bean seeds (*Pentaclethra macrophylla*), Roselle seeds (*Hibiscus sabdariffa*) and soy bean seeds (*Glycine max*) respectively [9-11]. Fermentation has been reported to improve the nutritional values, taste and aroma, and reduce/eliminate the anti-nutrient contents of these seeds [12].

Some indigenous fermented foods have enjoyed technological advancement by the use of proven strains as starter cultures [11,13]. The use of starter cultures is very important in

Microbiology. It helps to differentiate between the food proximate compositions, organoleptic properties of foods produced by natural fermentation and controlled fermentation. This will also ensure standardization of product in terms of shelf-life, stability, predictability and hygienic quality [14].

Lima bean is among the underutilised legumes which are widely available and thrive well in lowland tropical rain forest areas and on poor soils where most crops cannot grow well [8]. Lima bean contains about 22% crude protein content and the yield per hectare is between 3000kg and 5000kg of seeds [7].

However, Lima bean seeds contain anti-nutritional factors such as phytins, tannins, hydrogen cyanide and trypsin inhibitors which limit their usefulness as food [8]. Anti-nutritional compounds reduce food intake and nutrient utilization in animals and lower the nutrient value of grain legumes [15,16]. Besides it takes several hours usually overnight with several changes of cooking water before it could be edible [17]. Lima bean seeds have been fermented which resulted in increased protein and fat contents as well as reduction in their anti-nutrient contents [18]. Various genera of microorganisms were identified during the fermentation process, with *Bacillus* species being the most predominant [18].

The aim of this research was to study the effect of single starters of *Bacillus subtilis* and *B. pumilus* on the nutrient value, anti-nutrient contents as well as the organoleptic properties of ground-cooked Lima bean seeds.

2. MATERIALS AND METHODS

2.1 Preparation of the Samples

Lima bean seeds used for this research work were brought from Ayegunle-Ekiti, Ekiti State, Nigeria. The healthy seeds were sorted and washed with sterile distilled water, drained and soaked in hot water for 2 hours to enhance easy removal of the testa from the cotyledons. The cotyledons were washed and rinsed severally with sterile distilled water. The samples were cooked for 6 hours and finally dried using a drying cabinet at 57ºC for 48 hours.

One thousand two hundred grams of dried Lima bean cotyledons were washed with 2 litres of water containing 8g sodium metabisulphite (4%) and rinsed severally with sterile distilled water. The sample was ground using a blender sterilized with 90% ethanol and rinsed with sterile distilled water.

2.2 Inoculation Procedure

Pure cultures of *Bacillus subtilis* and *Bacillus pumilus* were collected from the Department of Microbiology, Adekunle Ajasin University, Akungba-Akoko, Nigeria. The starter cultures were previously isolated from naturally fermented Lima bean seeds [18] before they were transferred into nutrient agar slants. They were then subcultured from the slants into nutrient agar plates using streak plate technique. Inoculum was transferred from each plate of the organisms into 100ml of sterile saline water and incubated at 35ºC for 24 hours. Fifteen millilitres each of cell suspension (5%) containing 1.2×10^4 cell/ml was added separately to 300 g of the pretreated Lima bean cotyledons in duplicates. The samples were then wrapped with sterile banana leaves and placed in a sterile calabash previously sterilized with 75% alcohol and rinsed with sterile distilled water. The calabashes were then covered with their lids and allowed to ferment at ambient temperature (26±2ºC) for 9 days. Naturally fermented ground-cooked sample served as control.

2.3 Viable Cell Counts

Fermenting beans (5.0g wet wt.) were homogenized with 45 ml of sterile distilled water. Ten fold serial dilutions were made and 1.0ml of 10^{-4} dilution was inoculated into plate count agar plates (Oxoid CM 325) in duplicates using pour plate technique. Inoculated plates were incubated at 35ºC for 48 hours after which the bacterial colonies were counted on daily basis and expressed as colony forming units per gram (cfu/g) of the sample [19].

2.4 Temperature, Total Titratable Acidity and pH

The temperature of the fermenting paste was monitored on daily basis for 9 days by aseptically inserting a thermometer sterilized with 75% ethanol into the fermenting samples. The % total titratable acidity was determined by diluting 10g of the sample in 90 ml of sterile distilled water. The mixture was homogenized and allowed to settle from which 20ml was titrated against 0.1N NaOH using phenolphthalein as indicator. The pH was determined using a pH meter (Crison Basic model 20) calibrated with standard buffer (pH 7.0 and 4.0).

2.5 Proximate Composition

The proximate composition of the naturally fermented and starter culture fermented Lima bean seeds was determined according to the method described in AOAC [20]. The parameters determined include moisture, crude protein, crude fats, crude fibre, and carbohydrate contents.

2.6 Anti-Nutrient Determination

The raw and fermented Lima bean seeds were analysed for the presence of saponins cyanide, trypsin inhibitor, phytate, oxalate and tannin according to the methods described in Egwaikhide et al. [21], Bradbury et al. [22], Roy and Rao [23], Lucas and Markakes [24], Day and Underwood [25] and Van der Poel [26] respectively.

2.7 Sensory Evaluation

The fermented samples were served 20 untrained judges to evaluate the sensory qualities (odour, colour, texture, and overall acceptability) using a five-point hedonic scale (1 and 5 representing extremely dislike and extremely like, respectively) [11].

2.8 Statistical Analysis

Data obtained were analyzed by ANOVA and significant differences between means were compared using Duncan multiple range test with the aid of SAS/STAT program [27].

3. RESULTS

3.1 Bacterial Counts

The bacterial counts of the starter culture fermented samples as well as the naturally fermented sample increased till the fifth day of fermentation after which they started to reduce. Total bacterial count of *B. subtilis* fermented substrate increased from 4.65 to 9.34 log cfu/g and later reduced to 6.46 log cfu/g while *B. pumilus* fermented substrate increased from 4.17 to 10.23 logcfu/g and also declined to 6.28 at the end of the fermentation period. Bacterial counts increased from 5.06 to 8.14 log cfu/g in the naturally fermented sample after which it reduced to 5.91 log cfu/g (Fig. 1).

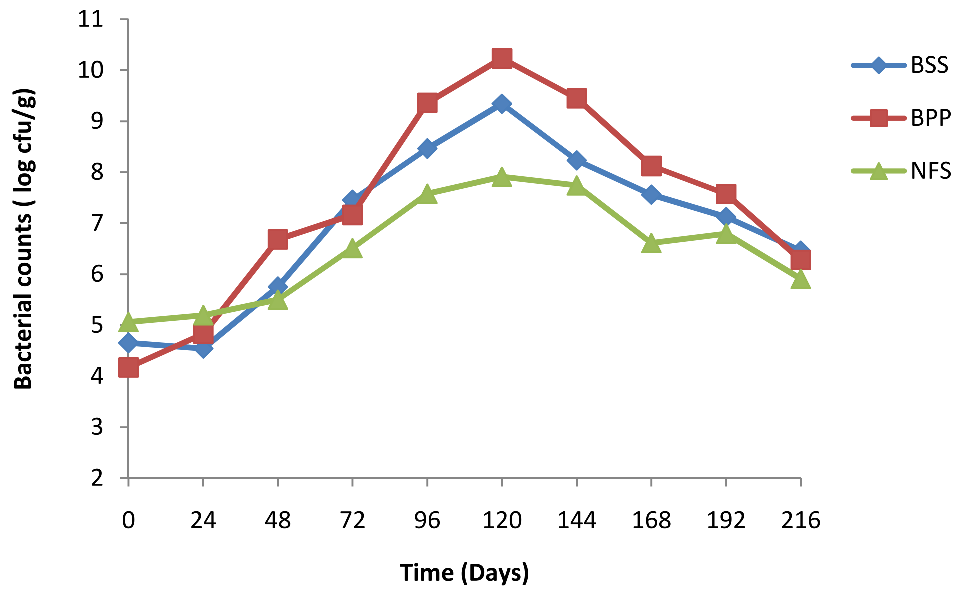

Fig. 1. Bacterial counts of the fermenting lima bean seeds

Legend: BSS= B. subtilis fermented sample; BPP= B. pumilus fermented sample; NFS= Naturally fermented sample

3.2 pH of the Fermenting Samples

The pH increased in all the fermenting samples (Table 1). The highest pH was observed in the sample fermented with *B. subtilis* (6.92) followed by *B. pumilus* sample (6.93) at the end of the fermentation while the natural sample had the lowest value (5.78) at 7th day of fermentation and was significantly lower than the those determined in starter culture fermented samples .

3.3 Total Titratable Acidities (TTA) of the Fermenting Samples

Total titratable acidities reduced in all the fermenting samples (Table 2). The lowest TTA was found in *B. subtilis* fermented sample (0.26%) followed by *B. pumilus* sample (0.25%) while naturally fermented sample had its lowest value at 7th day of fermentation (0.31%) and was also significantly lower than the those determined in starter culture fermented samples.

Table 1. pH of the fermenting samples

Sample code	Fermentation period (days)				
	1	3	5	7	9
BSS	5.51±0.11[a]	5.91±0.07[a]	6.23±0.04[a]	6.51±0.01[a]	6.92±0.02[a]
BPP	5.53±0.01[a]	5.87±0.02[ab]	6.23±0.02[a]	6.57±0.02[a]	6.93±0.01[a]
NFS	5.21±0.02[b]	5.60±0.03[b]	5.96±0.02[b]	5.78±0.03[b]	5.72±0.02[b]

Samples with the same superscripts down the column are not significantly different
Legend: BSS= B. subtilis fermented sample; BPP= B. pumilus fermented sample; NFS= Naturally fermented sample

Table 2. TTA of the fermenting samples

Sample code	Fermentation period (days)				
	1	3	5	7	9
BSS	0.46±0.02[ab]	0.40±0.01[bc]	0.30±0.02[b]	0.28±0.02[b]	0.26±0.03[b]
BPP	0.46±0.03[ab]	0.41±0.01[b]	0.31±0.02[b]	0.28±0.03[b]	0.25±0.04[b]
NFS	0.48±0.01[a]	0.43±0.02[a]	0.34±0.01[a]	0.31±0.02[a]	0.34±0.01[a]

Samples with the same superscripts down the column are not significantly different.
Legend: BSS= B. subtilis fermented sample; BPP= B. pumilus fermented sample; NFS= Naturally fermented sample

3.4 Temperature of the Fermenting Lima bean seeds

The fermentation was accompanied with initial increase in temperature. Ground Lima bean seeds fermented with *B. subtilis* and *B. pumilus* had the highest temperature of 33.8ºC and 32.8ºC respectively at 96th hour while the uninoculated sample had the highest increase of 29.2ºC at 168 hours of fermentation (Fig. 2).

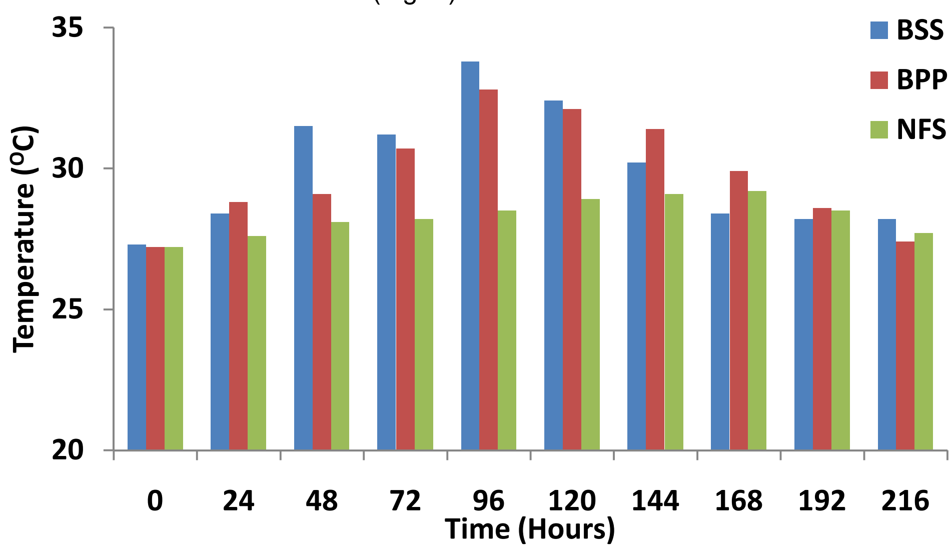

Fig. 2. Temperature of the fermenting lima bean seeds
Legend: BSS= B. subtilis fermented sample; BPP= B. pumilus fermented sample; NFS= Naturally fermented sample

3.5 Proximate Composition of the Fermenting Lima Bean Seeds

Fig. 3 shows the proximate composition of the samples during fermentation. Moisture contents increased throughout the fermentation period. Moisture contents of the sample fermented with single starters of *B. subtilis* and *B. pumilus* increased from 35.47% and 35.98% to 40.11% and 42.15% respectively while uninoculated increased from 35.82% to38.94%. Protein content increased till the fifth day of fermentation and later dropped. The respective protein contents for *B. subtilis*, *B. pumilus* and uninoculated samples at this optimum period were 24.32%, 25.54% and 22.22% .Fat contents increased throughout the fermentation period with the highest in *B. pumilus* fermented sample (3.31%), followed by the sample fermented with *B. subtilis* (2.63%) while the lowest content was found in uninoculated sample (2.22%). Ash contents of sample fermented with starter cultures increased from 4.23% to 4.39% and from 4.39% to 4.77% in *B. subtilis* and *B. pumilus* fermented samples respectively. Reductions were however observed in the ash content of naturally fermented sample (4.43-4.31%) after fermentation. The fibre contents of the Lima bean seeds fermented with *B. subtilis*, *B. pumilus* and uninoculated sample reduced from 5.23%, 5.33% and 5.83% to 3.39%, 3.27% and 4.01% respectively. Carbohydrate contents also reduced in all the samples after fermentation with the highest reduction in *B. pumilus* fermented sample (35.05 -25.22)% followed by *B. subtilis* fermented sample (35.05 -25.22)% while the naturally fermented sample had the highest content at the end of the fermentation (31.25-29.92)%.

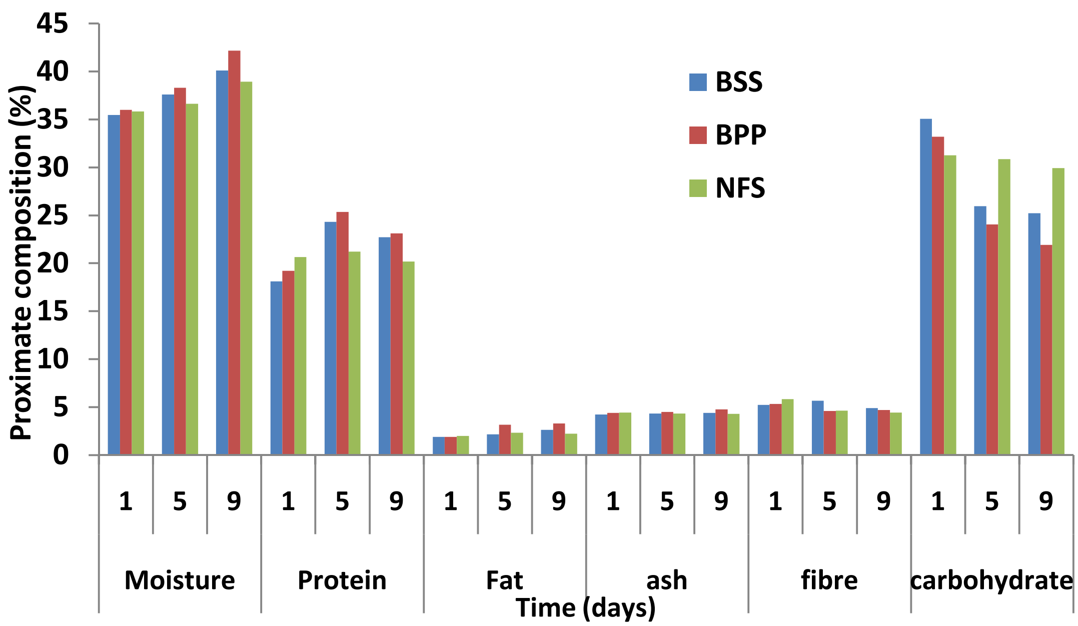

Fig. 3. Proximate composition of the fermenting lima bean seeds
Legend: BSS= B. subtilis fermented sample; BPP= B. pumilus fermented sample; NFS= Naturally fermented sample

3.6 Anti-Nutrient Contents of the Fermenting Lima Bean Seeds

Higher significant reductions (p<.05) were observed in all the anti-nutrient contents after fermentation (Table 3). Saponin was reduced from 1.50% in raw sample to 0.57%, 0.51% and 0.54% in sample fermented with *B. subtilis*, *B. pumilus* and uninoculated sample respectively. Ground unfermented Lima bean seeds contained 1.61mg/g oxalate content. When fermented with *B. subtilis* and *B. pumilus*, their oxalate contents of the samples were respectively reduced to 0.47mg/g and 0.45mg/g while the uninoculated sample dropped to 0.42mg/g. Similar trend was also observed in phytic acid contents of the sample reduced from 13.9mg/g in the raw sample to 3.64 mg/g, 3.51mg/g and 3.50mg/g in *B. subtilis*, *B. pumilus* fermented samples and uninoculated sample respectively. Tannin content reduced from 0.84mg/100g to 0.14mg/100g in both *B. subtilis* and uninoculated sample while *B. pumilus* fermented sample was reduced to 0.13mg/100g. Cyanide reduction was more pronounced in the uninoculated sample than the starter culture fermented sample. The highest cyanide content was observed in *B. subtilis* fermented sample (19.23 mg/100kg) followed by *B. pumilus* fermented sample (17.98mg/100kg) and uninoculated sample (16.75mg/100kg) all which were significantly lower than the content observed in unfermented sample (56.76 mg/100kg).

Table 3. Anti-nutrient contents of the fermenting lima bean seeds

Sample code	Saponin (%)	Oxalate (mg/g)	Phytic acid (mg/g)	Tannin (mg/100g)	Cyanide (mg/100Kg)
BSS	$0.57\pm0.0\ 2^{b}$	0.47 ± 0.02^{b}	3.64 ± 0.06^{b}	0.14 ± 0.01^{b}	19.23 ± 0.13^{bc}
BPP	0.51 ± 0.02^{bc}	0.45 ± 0.01^{bc}	3.51 ± 0.04^{b}	0.13 ± 0.01^{b}	17.98 ± 0.07^{bc}
NFS	0.54 ± 0.03^{b}	0.43 ± 0.03^{c}	3.50 ± 0.13^{b}	0.14 ± 0.02^{b}	16.75 ± 0.06^{c}
GUF	1.50 ± 0.02^{a}	1.61 ± 0.17^{a}	13.9 ± 0.02^{a}	0.84 ± 0.03^{a}	56.76 ± 1.89^{a}

Samples with the same superscripts down the column are not significantly different
Legend: BSS= B. subtilis fermented sample; BPP= B. pumilus fermented sample; NFS= Naturally fermented sample GUF= unfermented ground sample

3.7 Organoleptic and Physical Properties of the Fermenting Lima Bean Seeds

The starter culture fermented samples were rated best in all organoleptic parameters while fermented ground cooked sample was significantly lower (p<.05) than the two starter culture fermented samples (Table 4). Sample fermented with *B. subtilis* was rated best in terms of appearance (3.1) and texture (3.3) while *B. pumilus* fermented sample was rated best on sliminess (3.3) and odour (3.2).

Table 4. Organoleptic and physical properties of the fermenting lima bean seeds

Isolates	Appearance	Sliminess	Texture	Odour
BSS	3.1 ± 0.01^{a}	3.2 ± 0.02^{a}	3.3 ± 0.02^{a}	3.0 ± 0.02^{ab}
BPP	2.9 ± 0.02^{a}	3.3 ± 0.01^{a}	3.1 ± 0.02^{a}	3.2 ± 0.02^{a}
NFS	2.4 ± 0.05^{b}	2.8 ± 0.46^{b}	2.8 ± 0.26^{b}	2.8 ± 0.36^{b}

Samples with the same superscripts down the column are not significantly different
Legend: BSS= B. subtilis fermented sample; BPP= B. pumilus fermented sample; NFS= Naturally fermented sample

4. DISCUSSION

Bacillus species which were used as starter cultures in this research have been implicated in the fermentation of many leguminous and carbohydrate products. *Bacillus subtilis* was reported to be predominant while fermenting African locust beans *(Parkia biglobosa*) for *iru* [28], cotton seed (*Gossypium hirsutum*) for *owoh* [29], melon seed for *ogiri* [30], African oil bean (*Pentaclethra macrophylla*) for *ugba* [31] and *Prosopis africana* seeds for *okpehe* [32]. Adegbehingbe and Odunfa [33] observed the persistence of *B. polymyxa* during the production of *ogwo*, a fermented beverage produced from Irish potato and sorghum mixture. The use of a single starter or mixture of microorganisms with complimentary physiological properties seems to be a good approach for obtaining a product with the nutritional and sensory properties desired [34]. Controlled fermentation of soya bean, African mesquite and African locust bean seeds was achieved by using pure single culture of *B. subtilis, B. pumilus, B. licheniformis* or in combination [3,35]. The higher microbial counts in starter fermented products as observed in this study could be as a result of lack of competition and inhibitory compounds which could have otherwise hindered the fermenting organisms if fermented naturally.

The decreases in titratable acidities and subsequent increases in pH of the fermenting Lima bean seeds were similar to Omafuvbe [36] while fermenting soya bean seeds. Yagoub *et al.* [37] also reported decrease in acidity during *furundu* fermentation. This could be ascribed to the leaching of some of the acidic constituents into fermenting water. It could also be due to action of the microbial enzymes on substrate proteins, carbohydrates and lipids. Some *Bacillus* species have been reported to possess the ability to produce enzymes such as proteases, that breakdown protein to amino acids thus making the medium to become alkaline due to the presence of alkaline amino acids [38].

The increase in moisture contents of the fermenting Lima bean seed substrates agrees closely with the report of Omafuvbe et al. [39] while fermenting melon (*Citrullus vulgaris*) Seeds. The moisture content of the uncooked Lima bean seeds was 10.92%. The increase in moisture contents after cooking and of the Lima bean seed substrates could be due to absorption of water by the seeds during soaking and cooking and the subsequent increase during fermentation could be attributed to the decomposition of the substrates by the fermenting organisms which released moisture as one of their end products [28; 43].

The highest protein content observed in Lima bean seeds fermented with single starters of *Bacillus* species might be due to their ability to synthesise proteinase enzymes. The increased growth and proliferation of the microbial biomass could also be responsible for increases in fat and protein contents in the substrates fermented with starter cultures [40,41]. Gberikon et al. [3] observed that seeds of *Parkia africana* produced from mixed cultures of *B. subtilis* and *B. pumilus* fermented faster and contained higher protein and lipids values than seeds which were allowed to ferment naturally. It has been established [42] that *Bacillus* species implicated in oil bean seed fermentation are important producers of proteases. These extracellular proteases easily hydrolyze complex plant proteins to amino acids and short chain peptides, thereby causing an increase in total nitrogen content.

The increase in oil content of Lima bean seeds during and after fermentation was in agreement with Fadahunsi et al. [43] while fermenting bambara (*Voandzeia subterranean*) nuts for *iru* production The increase could be as a result of soaking and boiling which could further be enhanced by fermentation [43]. This increase might also be due to the fact that some microorganisms could produce microbial oil [44]. Also some *Bacillus* species have been reported to synthesise fats which could have been responsible for higher fat contents in samples fermented with *Bacillus* species. Besides, it might be due to the reports of Esenwah and Ikenebomeh [45] and Effiong and Umoren [46] that soaking, cooking and fermentation might have been responsible for cleavages of the protein–lipid or carbohydrate-lipid linkages facilitating their easy extraction. However, Osman [47] observed decrease in fat content while studying the effect of different processing methods on nutrient composition, antinutritional factors and *In vitro* protein digestibility of Dolichos lablab bean (*Lablab purpureus*) which he attributed to high lipolytic enzyme activity which breaks down the triglyceride to simple fatty acids sterol esters and polar lipids, during soaking cooking and fermentation.

The slight decreases in the ash contents of naturally fermented Lima bean seeds was in agreement with the results obtained by Esenwah and Ikenebomeh [45] while fermenting African locust bean seeds. Loss in ash contents may be due to leaching of soluble inorganic salts into the processing water during the fermentation period [46] or the fermenting microflora used it for their metabolism [48]. However, the increase in ash contents when fermented with *Bacillus* starters conforms with Jeff-Agboola and Oguntuase [13] while fermenting soy bean seeds for *soy iru* production using *Bacillus sphaericus.* Enujiugha [49] also observed increase in ash from 2.1 to 2.9% dry weight within 72 hours during oil bean fermentation. Effiong and Umoren [46] while processing horse eye bean (*Mucuna urens*) attributed the increase in ash content to the microbial destruction of antinutritional factors which bound some of these minerals. The higher ash contents for the controls suggest that minerals in these foods would be much more available than in the starter fermented samples. Regardless of treatments, legumes are better sources of nutrients than cereals, tubers and roots. When fermented with starters however, the increases observed could be due to biosynthetic mechanisms of the fermenting organisms. *Bacillus* species have been reported for its ability to synthesize divalent metals [50].

The loss of fibre during the fermentation of Lima bean seeds could be due to hydrolysis and leaching into fermentation medium or the microflora used it for their metabolism [28]. The decrease in carbohydrate in this study might be as a result of breakdown of carbohydrate [2]. General significant reductions ($p<.05$) in anti-nutrient contents which were observed after fermentation of the Lima bean seeds had been reported by many authors on other substrates. Effiong and Umoren [46] observed drastic reduction in anti-nutrient contents of horse eye bean (*Mucuna urens*) during fermentation. The subsequent reductions in all the anti-nutrient contents during the fermentation had been reported and attributed to further leaching and microbial activities [51]. The beneficial effects other microorganisms such as lactic acid bacteria in degrading anti-nutritive factors and natural toxins had been reviewed [52]. Chelule [53] reported that some starter cultures of lactic acid bacteria were able to reduce to some levels the phytic acid and phenolic compounds in amahewu, a traditional South African fermented food. Azeke et al. [54] reported that *Rhizopus oligosporus* contained phytase which is responsible for phytate degradation.

Saponins can affect animal performance and metabolism in a number of ways such as erythrocyte haemolysis, reduction of blood and liver cholesterol, depression of growth rate, bloat (ruminants), inhibition of smooth muscle activity and bloat (ruminants) [55]. Saponins have also been reported to alter cell wall permeability and therefore produce some toxic effects when ingested [56]. Saponins have been shown to bind to the cells of the small intestine thereby affecting the absorption of nutrients across the intestinal wall.

Bacillus subtilis has been identified as being capable of producing organoleptically acceptable final products in alkaline fermented legumes [57]. The highest odour produced with the use of *Bacillus* species was in agreement with Ogueke and Aririatu [31]. The odour could be due to their ability to produce ammonial compounds during degradation of proteins and amino acids [58]. The use of single and mixed *Bacillus* starters which resulted in the highest degree of sliminess and softness of the substrates was reported by Nwagu et al. [11]. The high significant aroma production in Lima bean seeds fermented with bacterial starters reported in this study is in agreement with Sanni et al. [59].

5. CONCLUSION

Present findings indicate that Lima bean seeds fermented with *B. subtilis* and *B. pumilus* significantly increased the protein and ash contents of the products. Mass production using starter cultures will definitely reduce chances of microbial contamination, product variation, food borne diseases and intoxication. This could also set the foundation for acceptability and mass production of fermented Lima bean seeds for both animal and human protein supplements thereby addressing the problem of protein malnutrition.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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