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Improving the Chemical Composition and Functionality of Cowpea (*Vigna unguiculata*) Seeds via Lactic Acid Fermentation

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ABSTRACT

Legumes, particularly cowpea (*Vigna unguiculata),* are an important source of nutrients, especially in developing countries. Despite being part of the staple diets of these populations, the consumption of cowpea is limited by the flatulence they produce. Natural lactic acid fermentation has proven to be an effective method to decrease flatulence-producing compounds. However, in order to use this method as a process on a large scale, it is fundamental to identify the microbial flora involved. When fermented seeds of *Vigna unguiculata* (cowpea) were analyzed microbiologically, it was found that the micro-organisms present were *Lactobacillus fermentum, Lactobacillus cellobiosus,* and *Lactobacillus plantarum.* On performing back-slopping or induced fermentation, there was a significant ($p \leq 0.05$) increase in the protein from 23.1% in raw sample to 25.5% in the fermented product, and a 57.3%, 53.3% and 38.9% respective decreases in oxalate, phytate, and tannin contents when compared to the raw sample. When cooking of the spontaneous and induced fermented samples was done, there was a reduction in the anti-nutritional components and thus increase in potassium and phosphorus contents of the samples from 385.67±0.56 mg/100g and 459.33±0.56 mg/100g in the raw sample to 417.00±0.00 mg/100g and 435.33±0.56 mg/100g in the product, for the spontaneous and induced fermented samples, respectively.

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There was also a significant ($p \leq 0.05$) reduction in the bulk density from 0.76 ±0.03 g/cm^3 in raw samples to 0.65± 0.01 g/cm^3 and 0.60± 0.01 g/cm^3 in spontaneous and induced fermented cowpea seeds, underscoring the usefulness of the fermented product in weaning diets formulation, with increases in the water absorption, oil absorption, and emulsifying capacities. All these results demonstrate that the lactic acid bacteria used for the induced fermentation (or back-slopping) can lead to a functional food with improved nutritional quality.

Keywords: cowpea seeds, lactic acid fermentation, chemical composition, functional properties.

1. INTRODUCUTION

It is generally acknowledged that legumes play an important role in the indigenous agricultural practices and diets of many developing countries and are a major source of dietary nutrients for many people (Enujiugha, 2005; Ghavidel, 2006). Legumes are known to belong to the family *Leguminosae,* which is probably the second most important source of food and fodder, next only to the family *Gramineae*- the cereal grains. Legumes include, but not limited to: Mucuna Bean, Lima Bean, African Oil Bean, African Locust Bean, African Yam Bean, Soybean, Bambara Groundnut, Groundnut, Pigeon Pea, Kidney Bean, Jack Bean, and Cowpea (Enujiugha and Ayodele-Oni, 2003). Cowpea is a good source of proteins, carbohydrates, some minerals, and vitamins. Commonly used form of cowpea is cooked cowpea alone or as an ingredient in porridges with cereals, roots, and tubers. It may be consumed in that form or as porridge with stew, vegetable oil or palm oil. Cooking of cowpea with cereals -like rice- gives the best complementarity of amino acids. Other common food uses of cowpea include processing to make akara (bean cake), moin moin (bean pudding), and gbegiri (bean soup) (Elegbede, 1998). However, its nutritive value is limited by the presence of several antinutritional and toxic substances, including oligosaccharides (especially raffinose, stachyose, and verbascose, which are contributory factors to the flatulence problem), phytates, polyphenols, and trypsin inhibitors capable of decreasing the bioavailability and limiting the digestibility of the nutrients (Egounlety and Aworh, 2003). Thus, to reduce these antinutritional factors, certain processes are employed. These include soaking, germination, dehulling, heating, and fermentation (Enujiugha, 2020).

Fermentation involves microorganisms that are not only catabolic, breaking down more complex substances like flatulence-causing indigestible oligosaccharides, such as raffinose, stachyose, and verbascose, into simpler sugars and sugar derivatives, but also synthesize several complex vitamins and other growth factors (Oguntimehin et al., 2023; Potter and Hotchkiss, 2007). However, given the variability inherent in the bacteria present at a given time, it is fundamental to identify the microflora responsible for the process. Microorganisms in legume fermentation act upon the seeds to bring about changes in the texture, aroma, color, taste, and nutritional composition (Enujiugha et al, 2008). LAB also improves the natural texture of products due to exo-polysaccharidase production and contributes to the aroma and flavor of fermented products. They acidify the food, resulting in a tangy lactic acid taste, and produce aromatic compounds from, for instance, amino acids upon bio-conversion (Adisa et al., 2024).

Most legume fermentations ultimately lead to production of condiments (Enujiugha, 2009; Chinma et al., 2023). The conditions for the production of such soup condiments depend on the nature of the substrate, the method adopted for the fermentation, and process variables such as temperature and pH. In this study, cowpea seeds were subjected to both natural and induced fermentation, and the effects on the innate seed components and its functionality were determined.

2. MATERIALS AND METHODS

2.1. Fermentation of Cowpea

Commercial cowpea seeds were bought from a local market (Oja Oba, Akure, Nigeria) and fermented in the proportion 1:4 (w/v) at 42 ºC for 48 h. Thereafter, the seeds were aseptically drained, and the fermentation water was characterized microbiologically and labeled the initial culture. This liquid was used in the induced fermentation of the next batch of cowpeas for 48 h. The liquid was again drained and microbiologically characterized. Samples from the raw, natural, and induced fermentation (both taken at 24 h and 48 h) were dried and used for further chemical analysis.

2.2. PH Determination

Ten grams (10 g) of sample taken at 24-hour intervals during fermentation was homogenized with 100 ml sterile distilled water and allowed to stand for 30 minutes. The mixture was then filtered, and the pH measured using a pH meter standardized with buffers at pH 4 and 7 at ambient temperature (AOAC, 2012)

2.3. Isolation and Characterization of the Fermentative Microorganisms

One milliliter (1 ml) of each sample was taken and serially diluted. This was then introduced asceptically into already prepared Nutrient Agar (NA) and de Mann, Rogosa, and Sharpe (MRS) agar by pour plating technique and incubating the NA plates in an inverted position at 37 ºC while the MRS plates were incubated at 42 ºC for 48 h in an anaerobic jar, after which the plates were observed for growth of mesophiles and lactic acid bacteria (Oguntimehin et al., 2023; Adejobi et al., 2024a; Adisa et al., 2024). Different microbial colonies were selected using a cooled, sterilized wire loop. This was transferred to already solidified plate of agar by streaking across the surface of the agar. The plates were incubated again at appropriate temperature and time. The pure isolates obtained were then transferred to already prepared slants for storage.

For preliminary identification, colonial characteristics of the isolated organisms on the solid agar were carried out for such traits as translucency, elevation, edges, and surface texture (Adejobi et al., 2024b). Cultural characteristics of the bacterial isolates were recorded using Olutiola et al (1991) methods, while the subsequent biochemical tests were carried out for the identification according to Holt et al (1994) methods.

2.4. Determination of Proximate Chemical Composition

The proximate analyses of the samples were carried out using the official methods of analysis of the Association of Official Analytical Chemists (AOAC, 2012) and replicated three times.

Moisture content was according to the air oven method (AOAC, 2012), whereby drying was done to constant weight; crude protein was determined using the micro-Kjeldhal method, and the total nitrogen in the sample was multiplied by a factor 6.25 (AOAC, 2012); crude fat was extracted overnight in a Soxhlet extractor with n-hexane and quantified gravimetrically; ash content was determined in the sample by dry ashing in a muffle furnace at 550 ^{o}C for 8 hours (AOAC, 2012); crude fibre was determined after digesting five grams (5 g) of fat-free sample in mixture of refluxing 1.25% sulphuric acid and 1.25% sodium hydroxide; and total available carbohydrates were determined by the difference method (subtracting the percent crude protein, crude fibre, crude fat, and ash from 100% dry matter). All analyses were carried out in triplicates. The energy values of the samples were obtained by multiplying crude protein, crude fat, and carbohydrate contents by factors of 4, 9 and 4, respectively (Enujiugha and Ayodele-Oni, 2003).

2.5. Determination of Mineral Content of the Samples

The mineral analyzed for included calcium, iron, potassium, zinc, magnesium, phosphorus, and sodium. The minerals were analyzed from the solution obtained by first dry ashing. Two grams (2 g) of each sample in a crucible was placed in the muffle furnace at 550 ^{o}C for 5 hours to ash and then transferred into a desiccator to cool. The cooled ash was dissolved in 10% HCl and filtered, then subsequently diluted to 50 ml volume in standard volumetric flask with deionized water. Sodium and potassium were determined using the flame photometric procedure, while phosphorus was determined colorimetrically by the phosphovanado-molybdate (yellow) method (AOAC, 2012). The remaining sample solution was aspirated into the atomic absorption spectrophotometer to obtain the concentrations of the other minerals (Fagbemi et al., 2024).

2.6. Determination of Tannins, Phytates, and Oxalates in the Samples

Phytic acid was extracted from each 3 g flour sample with 3% trichloroacetic acid by shaking at room temperature, followed by high-speed centrifugation. The phytic acid in the supernatant was precipitated as ferric phytate, and iron in the sample was estimated. Phytate-phosphorus (phytate-P) was calculated from the iron results, assuming a 4:6 iron: phosphorous molecular ratio (Enujiugha and Olagundoye, 2001). The phytic acid was estimated by multiplying the amount of phytate-phosphorous by the factor 3.55 based on the empirical formula $C_6P_6O_{24}H_{18}$. Tannin contents were determined by the modified vanillin-HCL method of Burns (1971). Determination of oxalate was by the AOAC (2012) method. All procedures were carried out in triplicates.

2.7. Determination of Functional Properties

The determination of water and oil absorption capacities followed a modification of the method of Enujiugha et al. (2003). Each flour sample (5.0 g) was thoroughly mixed, without pH adjustment, with 25 ml of deionized water or oil in 50-ml centrifuge tubes. Suspensions were stirred intermittently over a 30 min period at room temperature (25 ℃) and then centrifuged at 12,000 x g for 30 min at 25 ℃. The volume of decanted supernatant was measured, and the water and oil absorption capacities were then calculated. Triplicate samples were analyzed for each flour sample category.

Emulsifying properties were determined using a modification of the method described by Enujiugha and Akanbi, 2005). A known quantity (1.8 g) of sample was dispersed in 25 ml distilled water, and 25 ml vegetable oil (pure groundnut oil) was added.

The 50 ml mixture was emulsified at high speed using ultra-Turax T25 mixer for 1 min. Emulsion was filled into centrifuge tubes and centrifuged for 5 min at 1,300 x 6 rpm. Percentage emulsion was then expressed as % Emulsion = 100 x (height of emulsified layer) / (height of whole solution in centrifuge tube). The results were expressed in percentages (g / g basis).

Bulk density was determined as described by Gbadamosi et al. (2011), with slight modifications. A 50 g powdered sample (≤ 250 µm particle size) was put into a 100 ml measuring cylinder. The cylinder was tapped several times on a laboratory bench to a constant volume. The bulk density (gm^{-3}) was calculated as weight of sample flour (g) divided by flour volume (cm^3).

3. RESULTS AND DISCUSSION

3.1.The pH of Raw and Fermented Samples

The results of the pH of raw and fermented samples are presented in Table 1. There was a reduction in the pH of the raw cowpea on subjecting it to spontaneous (natural) fermentation in a progressive pattern for 48 h as a result of lactic acid produced by the fermenting microorganisms. Lactic acid bacteria (as probiotics) hydrolyze fibers, thus increasing the concentration of glucose and maltose, which are later transformed into organic acids and alcohols, causing a decrease in the pH of the fermenting medium (Ukeyima et al., 2010). The pH eventually falls to 4.76 which is higher than 4.5, a characteristic of lactic acid fermentation as indicated by Frais et al. (1996) and Adisa et al. (2024); and upon induced lactic acid fermentation, it decreased further, which is in line with the findings of Granito et al. (2003) who found a dramatic decrease in the pH of beans (*Phaseolus vulgaris*) fermented from 24-72 h with *L. acidophilus*, *Bifidobacterium* spp., and *Streptoccus thermophilus,* which are lactic acid bacteria.

3.2. Proximate Composition of the Samples

The result of the proximate composition of raw dehulled (RS), naturally fermented (NF), induced fermented (IF), naturally fermented and cooked (NFC) and induced fermented and cooked (IFC) cowpea seeds is presented in Table 2. Fermentation increased the protein and moisture contents, with higher increases in induced fermented cowpea and natural fermented cowpea, respectively. The increase in moisture content could be as a result of fermentation and cooking in water; thus, the seeds would have absorbed more moisture. The significant ($P \leq 0.05$) increase in protein from.

Table 1. The pH of raw and fermented samples.

Fermentation Time (h)	RS	NF	IF
0	6.377±0.012	6.377±0.012	4.757±0.006
24	-	4.917±0.012	4.413±0.015
48	-	4.760±0.015	4.317±0.015

RS: Raw sample; NF: Naturally fermented sample; IF: Induced fermented sample

Table 2. Proximate nutrient composition of the samples.

Sample	Moisture content (%)	Protein (%)	Fat (%)	Carbohydrate (%)	Crude Fibre (%)	Ash (%)
RS	5.02±0.02^{c}	23.10±0.35^{c}	1.53±0.00^{a}	66.50±0.13^{a}	0.91±0.11^{a}	2.93±0.02^{a}
NF	7.19±0.02^{a}	25.23±0.15^{a}	1.13±0.06^{b}	64.87±0.18^{c}	0.54±0.01^{b}	1.03±0.03^{c}
NFC	7.51±0.17^{a}	24.53±0.12^{b}	0.88±0.15^{c}	65.85±0.13^{b}	0.41±0.02^{c}	0.82±0.02^{d}
IF	5.42±0.01^{c}	25.50±0.00^{a}	1.15±0.01^{b}	66.26±0.04^{b}	0.58±0.02^{b}	1.10±0.01^{b}
IFC	6.71±0.54^{b}	24.80±0.00^{b}	1.02±0.02^{c}	65.93±0.55^{b}	0.58±0.01^{b}	1.01±0.01^{c}

Values within each column with common alphabets are not significantly different at p≥0.05

RS: Raw sample
NF: Naturally fermented sample
NFC: Naturally fermented and cooked sample
IF: Induced fermented sample
IFC: Induced fermented and cooked sample

23.10% in raw cowpea to 25.2% in naturally fermented cowpea is within the range of 24.10% which Otunola and Obisesan (2004) obtained when *Vigna unguiculata* was fermented using *Rhizopus oligosporus*; and also in agreement with findings of Enujiugha (2003), who reported an increment in seed product protein content during the fermentative process of *Pentaclethra macrophylla* (Benth) seeds. This increase in protein content of fermented cowpea appears to agree with previous studies, especially on soybeans (Otunola et al., 1990) suggesting that fermentation can improve the nutritional status of legumes.

It was also observed that the ash content in the raw sample reduced from 2.93% to 1.027%, 0.82%, 1.098% and 1.01% in naturally fermented, naturally content as compared to the raw and fermented samples only, with the naturally (spontaneously) fermented cowpea seed sample being higher in moisture content. There was also a decrease in crude fiber from 0.91% in raw sample to 0.5%, 0.41%, 0.58%, and 0.58% in naturally fermented, naturally fermented and cooked, induced fermented, induced fermented and cooked samples, respectively. This may be due to the production of extra-cellular enzymes that use cellulose and hemi-cellulose fermented and cooked, induced fermented, and induced fermented and cooked samples, respectively. The Ash content is important in food for various reasons; for example, it is an index of the quality of food materials used for animal feeding. The lower the values, the less useful it is for animal feeds. The low values obtained might be due to the removal of the ash (mineral)-containing components in the seed coats during the fermentation and subsequent hydrothermal (cooking) steps/processes (Enujiugha et al., 2003). The ash content is an approximation of the total minerals in foods. The increase in raw protein in fermented samples could be due to the protein hydrolysis produced by extra-cellular enzyme of fermentative micro-organisms, such as proteases, producing an increase in the total nitrogen content caused by the release of amino acids and short chain peptides. There was significant ($P \leq 0.05$) decrease in the fat, carbohydrate, crude fiber, and crude ash in the both the naturally fermented cowpea and induced fermented cow In the fermented and cooked samples, it was also observed that there was an increment in moisture during fermentation; and also, to hydrolysis and subsequent solubilization of some pectic compounds in the cooking water. At the end of the boiling process, the cooking water was more viscous than it was at the beginning of the process. This is an indication of the presence of mucilaginous materials in the cooking, which explains in part the reduction of the crude fiber content of cooked cowpea seeds.

There was also a reduction in the carbohydrate content of raw cowpea from 66.50% to 64.87%, 65.85%, 62.26%, and 65.93% in naturally fermented, naturally fermented and cooked, induced fermented, and induced fermented and cooked cowpea samples, respectively. This could be due to gelatinization and solubilization of starch in the cooking water. This decrement is in line with the findings of Granito et al. (2003) and Martin et al. (2004) for fermented *Phaseolus vulgaris* seed.

3.3. Mineral Composition of Samples

Table 3 shows the results obtained for the determination of some minerals present in the raw and fermented samples. There was reduction in calcium by 9.69%, 13.57%, 5.53%, 15.51% and in magnesium content by 15.03%, 22.72%, 22.72%, 7.70% and 1.05% in naturally fermented, induced fermented, naturally fermented and cooked, and induced fermented and cooked cowpea, respectively. This is due to the leaching of the minerals in fermentation and cooking water. This is in line with the work of Granito and Alvarez (2006) on fermentation of black beans, and Oguntimehin et al. (2023) on fermentation of locus bean seeds.

Table 3. Mineral element composition of the samples.

Samples	Ca (mg/100g)	Mg(mg/100g)	Na(mg/100g)	K (mg/100g)	P (mg/100g)
RS	120.33±0.58[a]	95.33±1.16[a]	309.67±0.58[e]	385.67±0.58[e]	459.33±0.58[e]
NF	108.67±0.58[c]	81.00±0.00[d]	371.00±1.00[c]	417.00±0.00[c]	502.00±1.00[c]
NFC	104.00±0.00[d]	73.67±0.58[e]	325.00±1.00[d]	411.67±0.58[d]	491.33±1.53[d]
IF	113.67±0.58[b]	88.00±0.00[b]	384.00±0.00[a]	435.33±0.58[a]	532.00±1.00[a]
IFC	101.67±0.58[e]	85.33±0.33[c]	375.33±0.58[b]	423.00±0.00[b]	516.67±1.16[b]

Values within each column with common alphabets are not significantly different at p≥0.05

RS: Raw sample
NF: Naturally fermented sample
NFC: Naturally fermented and cooked sample
IF: Induced fermented sample
FC: Induced fermented and cooked sample
IFC: Induced fermented and cooked sample

Table 4. Concentrations of anti-nutritional factors in the samples (mg/100g dry wt.).

Samples	Oxalate	Phytate	Tannin
RS	7.38±0.01a	20.60±0.5a	0.09±0.01a
NF	6.03±0.52b	14.83±0.3b	0.07±0.01b
NFC	1.89±0.01d	4.12±0.07d	0.03±0.00c
IF	3.15±0.01c	9.61±0.04c	0.06±0.01b
IFC	1.23±0.52d	2.20±0.01e	0.03±0.00c

Values within each column with common alphabets are not significantly different at p≥0.05

RS: Raw sample
NF: Naturally fermented sample
NFC: Naturally fermented and cooked sample
IF: Induced fermented sample
FC: Induced fermented and cooked sample
IFC: Induced fermented and cooked sample

Increase in the content of Na, K, P was due to reduction in anti-nutrient component during fermentation (that would have tied the minerals in fermented and cooked samples). This trend was also reported for legume-supplemented ogi fermentation (Enujiugha, 2006). The induced fermented samples had the highest values.

3.4. Anti−Nutritional Factors in the Samples

Table 4 shows that there was a significant reduction in the anti-nutrients contents of raw cowpea on fermentation. For the naturally fermented sample, by a 18.7%, 28% and 15.6% reduction in oxalate, phyate and tannin contents, respectively, when compared to the raw sample, while for the induced fermented sample, there was a 57.3%, 53.3% and 38.9% decrease in oxalate, phytate, and tannin contents, respectively, when compared to the raw sample. For the naturally fermented and then cooked sample, there was great decrement in oxalate, phytate, and tannin contents at 57.3%, 79.9%, 64.4%, respectively, when compared to the raw sample while the induced fermented and cooked sample showed 83.3%, 89.3%, and 70% decreases in oxalate, phytate, and tannin contents, respectively. The reduction in phytic acid could be due to leaching out of phytate in the fermentation water. Tannin content was also reduced during fermentation and cooking. This is in agreement with the work of Granito and Alvarez (2006) when black beans was fermented and then cooked, there was a 83% decrease in the tannin content. Generally, processing techniques reduce the level of anti-nutritional factors in oil seeds and legumes, as previously reported by Enujiugha et al. (2003) and Enujiugha and Ayodele-Oni (2003).

3.5. Functional Properties of the Samples

Table 5 shows that fermentation significantly increased the water absorption capacity (WAC) of the naturally fermented and the induced fermented samples, with that of the induced fermented sample having the highest value. Cooking of the fermented sample reduced the value obtained in the fermentation, though the values were still higher than that of the raw sample. An increase in WAC on fermented cowpea could be attributed to an increase in protein content and change in the quality of protein upon fermentation and breakdown of polysaccharide molecules due to the activity of amylase; hence, site for interaction with water and holding water would be increased. This is desirable in the production of some food products like moin-moin (bean pudding).
Fermentation also increased the oil absorption capacity (OAC) of naturally fermented cowpea, while it remained unchanged for the naturally cooked, induced fermented, and induced fermented and cooked samples. Increases in OAC could result into making available amino acids by unmasking non-polar residues and this enhances flavour retention and mouth-feel (Sosulski et al., 1976; Enujiugha et al., 2003).
Emulsifying activity (EA) of all samples fermented (naturally and induced) and cooked were increased as compared to the raw cowpea seeds. This improvement in EA by fermentation and cooking may be due to the dissociating and partial unfolding of polypeptides that exposed the hydrophobic sites of amino acids, which aided hydrophobic association of the peptide chains with the lipid droplets, so that the net result was that much greater volume/surface area of protein was made available, and emulsification capacity was enhanced (Enujiugha and Akanbi, 2005).
Fermentation also reduced Bulk density of the fermented and cooked samples, making it suitable for preparation of weaning foods formulation (Elkhalifa and Bernhardta, 2009). Reduction in bulk density means that the food would have less difficulty during passage along the gastrointestinal tract (GIT), with attendant less bulk in the colon.

Table 5. Functional properties of the different samples.

Samples	BD (g/cm^3)	WAC (%)	OAC (%)	EC (m/v %)
RS	0.76±0.03^a	23.33±0.33^d	20.00±0.00^b	50.01±0.03^e
NF	0.65±0.65^b	34.00±0.00^a	25.00±0.00^a	51.08±0.12^c
NFC	0.63±0.00^c	28.50±0.29^c	20.00±0.00^b	55.56±0.00^a
IF	0.60±0.01^d	33.50±0.29^a	20.00±0.00^b	51.06±0.00^d
IFC	0.59±0.00^d	30.00±0.00^b	20.00±0.00^b	53.33±0.00^b

Values within each row with common alphabets are not significantly different at p≥0.05

RS: Raw sample
NF: Naturally fermented sample
NFC: Naturally fermented and cooked sample
IF: Induced fermented sample
FC: Induced fermented and cooked sample
IFC: Induced fermented and cooked sample

Table 6. Total Viable Counts of Raw and Fermented Cowpea samples (cfu/g).

Samples	Fermentation Period	Mesophilic Bacteria	Lactic Acid Bacteria
RS	0 h	8×10^5	Nil
NF	48 h	1.05×10^7	1.19×10^7
IF	48 h	1.10×10^6	1.26×10^9

RS: Raw sample
NF: Naturally fermented sample
IF: Induced fermented sample

3.6. Total Viable Microbial Counts in the Samples

The total viable counts, as presented in Table 6, show an increase in mesophilic organisms in natural fermented cowpea from 8.6×10^5 cfu/ g in the raw sample to 1.05×10^7 cfu/ g in naturally fermented sample, and a further reduction on induced fermentation to 1.10×10^6 cfu/ g, because the fermenting organisms probably reduced the mesophiles or other organisms by suppressing their growth through the acid that was produced, making the environment not conducive enough for their proliferation (Granito and Alvarez, 2006).

At the same time, the lactic acid bacteria increased as the hours of fermentation increased, with the induced fermented sample having a higher number. This result also agrees with the lactic acid fermentation of black beans by Granito and Alvarez (2006), who noticed a rise in population of lactic acid bacteria after 48 hours, reaching a population density of 10^7 cfu/ g. This trend also agrees with the general pattern of lactic acid fermented foods (Ukeyima et al., 2010).

3.7. Micro-Organisms Isolated in Raw and Fermented Samples

The list of fermentative microorganisms, after morphological and biochemical characterization of the isolates from the raw, naturally fermented, and induced fermented cowpea seeds is presented in Table 7. Before fermentation, different micro-organisms co-existed on the substrate. The microorganisms found on the raw cowpea seeds were *Staphylococcus aureus*, *Escherichia coli*, *Aeromonas aerogenes*, *Bacillus subtilis*, and *Aerococcus* sp. The isolated *Escherichia coli* may have been from in the manure or soil that was used in planting the beans, since the organism indicates faecal contamination. *Bacillus subtilis*, common ubiquitous saprophytic mesophilic bacteria, might have been recovered from air, soil, water, and decomposing plant residue (Enujiugha et al., 2008; Oguntimehin et al., 2023).

During the course of fermentation, most of the mesophlic organisms isolated in the raw sample were suppressed by the lactic acid bacteria. The ability of most lactic acid bacteria species to secret metabolites that destroy common pathogens is an established scientific fact. This could have been the case in the present study. The organisms isolated in fermented cowpea were *Lactobacillus fermentum, Lactobacillus cellobiosus, Lactobacillus plantarum, Proteus vulgaris,* and *Corynebacterium fascians*. This agrees with the findings of Granito and Alvarez (2006) who isolated *Lactobacillus plantarum* and *Lactobacilus casei* from fermented black beans. *Proteus vulgaris,* isolated in the spontaneous (natural) fermentation of cowpea, may have come from the water used in the fermentation of the beans or through handling and utensils used in the processing.

4. CONCLUSION

It can be concluded from the results of the present study that induced lactic acid fermentation of *Vigna unguiculata* beans decreases anti-nutritional components, increases nutritional value, and functional properties of the beans, making it suitable for weaning diets. Likewise, the cooking applied after induced fermentation produced an additional diminution of the anti-nutritional components in the beans. More research works are recommended on the kinetics of lactic acid fermentation of cowpea seed so as to increase the nutritional and functional properties of diets of developing countries, especially weaning diet. The lactic acid bacteria involved in the bean fermentation, which included *Lactobacillus fermentum,* a known probiotic, could be used as functional starter cultures in food industries.

Tables 7. Occurrence of Micro-organisms in the samples during fermentation.

Samples	Fermentation Period (h)	Probable Occurrence
RS	0	1. *Staphylococcus aureus* 2. *Escherichia coli* 3. *Aeromonas aerogenes* 4. *Bacillus subtilis* 5. *Aerococcus sp.*
NF	48	6. *Lactobacillus fermentum* 7. *Lactobacillus cellobiosus* 8. *Lactobacillus plantarum* 9. *Proteus vulgaris*
IF	48	10. *Lactobacillus fermentum* 11. *Lactobacillus cellobiosus* 12. *Lactobacillus plantarum* 13. *Corynebacterium fascians*

RS: Raw sample

NF: Naturally fermented sample

IF: Induced fermented sample

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