Full Length Research

Effect of fermentation on sorghum and cowpea flour blends

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This study was designed to investigate the suitable proportions of cowpea that can be used to improve the nutrient content of sorghum and also to ascertain the effect of fermentation on the sample blends. The raw and fermented sample blends were analyzed for microbial load, proximate composition, mineral and anti-nutrient contents. The microorganisms isolated were Staphylococcus aureus, Bacillus cereus, B. polymyxa, B. licheniformis, Lactobacillus fermentum, L. acidophilus, L. plantarum, Streptococcus lactis, Aspergillus fumigatus, A. niger, Mucor mucedo and Rhizopus nigricans. Moisture content of unfermented sample blends ranged between 0.75 and 1.07% while that of fermented blends ranged between 26.96 and 42.65% respectively. Unsupplemented cowpea recorded the highest level of ash content before and after fermentation but crude protein increased after fermentation. Unsupplemented cowpea recorded the highest level of protein content. Cowpea: sorghum (7:3) also had a significant amount of protein when compared with sorghum: cowpea (8:2). Carbohydrate content reduced after fermentation while anti-nutrient content reduced significantly after fermentation process. There was significant increase in protein content of sorghum supplemented with cowpea, and a drastic reduction in the anti-nutrient content of all the fermented sample blends. Therefore, it can be concluded that sorghum supplemented with cowpea, then fermented for 72 h could be recommended for improving the protein quality of sorghum.

Key words: Sorghum, fermentation, anti-nutrient, Lactobacillus fermentum, cowpea.

INTRODUCTION

Sorghum (Sorghum bicolor) is a staple cereal based food that has been reported to be a major source of energy in most African’s diet (Elkhier and Hamid, 2008). Smith and Frederiksen (2000) and FAO (2005) also documented that sorghum is the 5th most important grain crop after wheat, maize, rice and barley which belongs to a member of the family Poaceae. It is a drought tolerant crop that provides a good source of energy and antioxidant (Taylor et al., 2006; Duodu et al., 2003). Sorghum thrives on a wide range of soils from light loams to heavy clays but

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grows well on light sandy soils (Kimber, 2000), tolerates a range of soil acidity from pH 5.0-8.5 and has a moderate tolerance to salinity (Cothren et al., 2000). Sorghum is composed of mainly starch, about 75-79% of grain weight, comprised of 70-80 amyllopectin and 20-30% amylose (Waniska et al., 2004). The pericarp and germ of sorghum grains are rich in minerals such as iron, zinc, potassium, phosphorus, dietary fibre, B vitamins and essential fatty acids such as linoleic acid (49%), oleic (31%), palmitic (14%), linolenic (2.7%) and stearic (2.1%) (FAO, 1995; USDA, 2014) which is sometimes lost during dry and wet milling processes (Taylor, 2003).

Sorghum is used in the production of different food varieties such as: bread, porridge, pancakes, muffins, dumplings and breakfast cereals like ogi (Taylor, 2003). It contains more fat than wheat and rice but slightly less than corn. Sorghum is a very important food crop because it is gluten-free which makes it an excellent replacement for people that are allergic to gluten intake (Farmcrowdy, 2017).

Cowpea (Vigna unguiculata [L] Walp) has been reported to be the most important food legume in the dry savanna of tropical Africa (AATF, 2005). It is consumed by millions of people in the tropics, especially Africa (AATF, 2005). Cowpea is very rich in protein and contains almost as much energy by weight as cereal grains (USDA, 2014). This has however made it a good compliment to fortify weaning foods such as sorghum. The technique employed in traditional weaning food formulations include the use of composite foods made from cereal and legumes such as cowpea (Sefa-Dedeh et al., 2001). It is also used in the preparation of various foods such as “akara” (a fried cowpea paste), “moi-moi” (a steamed cowpea paste) and “kpejigaou” (a griddled cowpea paste) (Phillips et al., 2003; Amonsou et al., 2008). Cowpea contains an average of 24 g protein/100 g and 7 g lysine/100 g protein (Phillips et al., 2003). According to FAOSTAT (2015), Nigeria is the World’s largest producer of cowpeas followed by Niger.

The protein inherent to cowpeas is located in the cotyledons while the minerals are concentrated on the seed coat (Adebooye and Singh, 2007). Cowpeas are majorly cultivated for human consumption in sub-saharan Africa countries, but it can also be used as animal feed, and raw material for processing green manure used to improve soil fertility (Singh et al., 2003, 2011). The fresh green seeds can be roasted as snacks for human consumption and it can also be used to make soups and a variety of delicacies. The dried seeds can also be used to prepare soup such as “gbegiri” (Onyenekwe et al., 2000). Cowpea is a nitrogen fixing plant which makes the soil more conducive for the cultivation of vegetables and other staple foods (Singh et al., 2003). However, despite the high content of cowpea, it contains some indigestible sugars such as refinose and stachyose (Onyenekwe et al., 2000) which produces flatulence when consumed.

Soaking and blanching has been documented to reduce the levels of these indigestible sugars that inhibits iron and calcium absorption (Hotz and Gibson, 2007; Lestienne et al., 2005). However, fermentation have been documented to improve protein digestibility and food quality in terms of increase in amino acids and vitamins production. Hotz and Gibson (2007) also reported that fermentation improves food safety and confers microbial stability in the fermented food product. Therefore, the objective of this study was to evaluate the effect of fermentation on the proximate composition, mineral content, anti-nutrient contents, physicochemical properties and microbial characteristics of sorghum-cowpea flour blends as well as the general acceptability through the development of a fortified food product.

MATERIALS AND METHODS

Source of materials

Dry sorghum (S. bicolor) and cowpea (V. unguiculata [L] Walp) grains were purchased from Oja-oba, a local market in Akure, Ondo state, Nigeria. The samples were transported to the laboratory in clean low density polythene bags.

Preparation of samples

Grits and stones were sorted to remove extraneous materials, after which the sorghum-cowpea samples were divided into four (4) portions, coded A, B, C and D. Portions A and B were 500 g of whole cowpea and sorghum respectively, portion C was a ratio of cowpea and sorghum (7:3), while portion D was a ratio of sorghum and cowpea (8:2). Each of the samples was mixed with 100 ml distilled water inside a 250 ml clean plastic container labeled A-D. These containers were taped at the edges and subjected to spontaneous fermentation for 72 h at room temperature. At the end of fermentation process, the samples were dried in an hot air oven at 65°C for 24 h and then packaged in low density polythene pouches and stored at 8°C prior to further analyses.

Proximate composition

The moisture, crude protein (Marcokjeldahl method), ash, crude fat, crude fibre, carbohydrate, mineral contents of samples were analyzed before and after 72 h of fermentation using the method described by the Association of Official Analytical Chemists (AOAC, 2012). The total carbohydrate content was calculated by difference method (subtracting the sum of % moisture, crude protein, crude fat and ash from 100%).

Mineral contents

5 g of sample were heated in a muffle furnace until white-grey ash powder was obtained. The ash powder was allowed to cool. 20 ml of distilled water and 10 ml of diluted hydrochloric acid was added to the ash powder. The mixtures were analyzed for heavy metals such as: potassium (K), sodium (Na), magnesium (Mg) and lead (Pb) using atomic absorption spectrophotometer; Bulk Scientific
Colonies were determined by titrating 10 ml of
the raw (control) and fermenting blend of sorghum and cowpea flour blends is shown in Table 1. The microbial population (cfu/g) of the total aerobic bacteria was determined using nutrient agar (NA) (Merck, Darmstadt, Germany) while De Man, Rogosa and Sharpe (MRS), (Merck) and M17 agar media (Oxoid, Basingstoke, Hampshire, England, UK) was used for the isolation of lactic acid bacteria (LAB). Four different colonies were randomly picked using visual assessment from the highest dilution factor of MRS and M17 agar plates to determine the dominant bacteria during the fermentation of the blends. All the samples were analyzed by homogenizing 1g of the fermenting blend with 9 ml sterile 0.1% buffered peptone water (BPW) (Merck) followed by appropriate dilutions, spread plating and incubation at the required temperatures. The NA agar plate were incubated at 37°C for 24 h while MRS agar plates were incubated anaerobically using anaerobic jar together with anaerocult system (Merck) at 37°C for 48 h. Colonies were selected randomly, purified and subjected to various biochemical tests such as: motility, spore staining, citrate, catalase, coagulase, etc. and sugar fermentation tests which includes: glucose, lactose, mannitol, etc according to the method employed by Ojokoh et al. (2015).

Statistical analyses

Analysis of variance (ANOVA) was performed on the data at p≤0.05 using MINITAB statistical software (Minitab® Release 14.13, Minitab Inc., USA). Significant means were separated using the least significant difference (LSD) at ps0.05.

RESULTS

Proximate composition

The proximate composition of fermented and unfermented sorghum and cowpea flour blends is shown in Table 1. Moisture contents of sorghum and cowpea samples before fermentation (control) was 0.75 and 0.83% for 30 min for a blood red colour to develop. 0-10 ppm standard saponin solutions was prepared from saponin stock solution. The standard solutions were treated similarly with 2 ml of 5% FeCl₃ solution. The absorbances of the sample as well as standard saponin solutions were read after colour development on a Spectronic 21D Spectrophotometer at a wavelength of 380 nm (AOAC, 2012).

Physico-chemical properties

The method described by AOAC (2012) was used to determine the pH and titratable acidity of the fermenting medium. Samples were taken every 24 h during the fermentation period using the method described by Fayemi and Ojokoh (2014). The pH of the samples was determined using an Orion pH meter (Model 310, Orion Research Inc, Beverly, MA) equipped with glass electrode. The titratable acidity (TTA) was determined by titrating 10 ml of thoroughly mixed sample against 0.1 M NaOH, using phenolphthalein as an indicator. Values obtained were expressed as % lactic acid. All analyses were carried out in triplicate.

Microbial characteristics

The microbial profile of the raw (control) and fermenting blend samples were determined at 24 hr interval. The changes in microbial population (cfu/g) of the total aerobic bacteria was determined using nutrient agar (NA) (Merck, Darmstadt, Germany) while De Man, Rogosa and Sharpe (MRS), (Merck) and M17 agar media (Oxoid, Basingstoke, Hampshire, England, UK) was used for the isolation of lactic acid bacteria (LAB). Four different colonies were randomly picked using visual assessment from the highest dilution factor of MRS and M17 agar plates to determine the dominant bacteria during the fermentation of the blends. All the samples were analyzed by homogenizing 1g of the fermenting blend with 9 ml sterile 0.1% buffered peptone water (BPW) (Merck) followed by appropriate dilutions, spread plating and incubation at the required temperatures. The NA agar plate were incubated at 37°C for 24 h while MRS agar plates were incubated anaerobically using anaerobic jar together with anaerocult system (Merck) at 37°C for 48 h. Colonies were selected randomly, purified and subjected to various biochemical tests such as: motility, spore staining, citrate, catalase, coagulase, etc. and sugar fermentation tests which includes: glucose, lactose, mannitol, etc according to the method employed by Ojokoh et al. (2015).

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Table 1. Proximate composition of fermented and unfermented sorghum and cowpea flour blends.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Component (%)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Moisture</td>
</tr>
<tr>
<td></td>
<td>Raw</td>
</tr>
<tr>
<td>Cowpea</td>
<td>0.83±0.06a</td>
</tr>
<tr>
<td>Sorghum</td>
<td>0.75±0.14a</td>
</tr>
<tr>
<td>Cowpea:sorghum (7:3)</td>
<td>1.07±0.03b</td>
</tr>
<tr>
<td>Sorghum: cowpea (8:2)</td>
<td>1.03±0.07a</td>
</tr>
</tbody>
</table>

Data are presented as Mean±S.E (n=3). Values with the same superscript letter(s) along the same column are not significantly different (P<0.05).

respectively. Unfermented samples had significant low moisture content (p≤0.05) compared to fermented blends of sorghum and cowpea. After fermentation, the sample blends had moisture content ranging from 26.96 to 39.94% (Table 1). However, the moisture content of all the samples were significantly different (p≤0.05) from each other. Therefore, it can be deduced from this results that moisture contents of fermented sorghum and cowpea blends were significantly higher than the unfermented samples. This observation could be attributed to the effect of soaking the samples during fermentation and the temperature of the fermenting medium. This observation is contrary to the report of Wakil and Kazeem, 2012; Momanyi, 2014; Ojokoh et al., 2014) in which ash content during the fermentation of sorghum and cowpea blends ranged from 0.505 to 0.67% was observed after the fermentation of sorghum and cowpea blends. However, the ash content decreased significantly after the fermentation of sorghum and cowpea blends. Ash content ranging from 0.505 to 0.67% was observed after the fermentation of sorghum and cowpea blends. There was no significant (p≤0.05) in the ash content of fermented sorghum (0.57%) and fermented sorghum-cowpea (8:2) blends (0.50%). This observation is in contrast with the report of Ojokoh et al. (2014) in which ash content increased as the amount of cowpea increases during the fermentation cowpea-breadfruit blends. The ash content ranged from 2.42 to 3.61%. The total ash content of fermented sorghum (0.57%) and sorghum-cowpea blends (8:2) (0.50%) were not significantly (p≤0.05) different. The decrease in ash content as observed in this study could be attributed to the general activities of the fermenting microorganisms whose enzymatic activities has been broken down into absorbable forms. Crude fat of raw unfermented sample blends ranged from 0.88 to 2.07%. The fat content of raw cowpea was significantly (p≤0.05) higher (2.07%) compared to the blend samples of cowpea and sorghum (7:3) (1.77%). As fermentation increases, the fat content of sorghum and cowpea (8:2) blends decreases (0.41%)...
No significant difference was observed in the fat content of fermented sorghum and sorghum-cowpea (8:2) (0.43 and 0.41%) respectively. Low percentage of crude fat observed in this study signifies prolong storage of the food blends. High fat content in foods causes rancidity which could impact unpleasant odor in the food (Ikram et al., 2010). The result of this work is contradictory to the earlier report of Ojokoh et al. (2014) who observed a significant increase in the proportion of fermented cowpea-breadfruit blends (3.05 to 4.72%). The crude fiber content of unfermented sorghum and cowpea blends ranged from 2.05 to 3.43% compared to 1.09% for cowpea and 4.90% for sorghum. Crude fiber content of fermented sample blends were significantly different at (p≤0.05). Fermented sorghum blends (8:2) had a higher fiber content (2.05 and 1.27%) respectively than fermented cowpea and cowpea-sorghum blends (7:3), 0.75 and 0.89% respectively. This report disagrees with the findings of Ojokoh et al. (2014) who observed a significant increase in ash content during the fermentation of cowpea-cereal blends.

Mineral composition

Table 2 shows the mineral compositions (100/mg) of unfermented sorghum, cowpea, cowpea-sorghum blends (7:3) and sorghum-cowpea blends (8:2). The potassium (K) compositions of raw unfermented cowpea, sorghum, cowpea-sorghum (7:3) and sorghum-cowpea (8:2) blends were 320, 448, 434 and 444% respectively while that of fermented sample blends were 287.50, 390.50, 403.50 and 395.50% respectively. The sodium (Na) compositions of raw unfermented blends were 23.50, 30.50, 39.50 and 30.00% respectively while that of fermented samples was 11.00, 20.50, 25.00 and 30.00% respectively. However, the magnesium (Mg) compositions of unfermented samples were 259.00, 290.00, 270.50 and 321.50% respectively while that of fermented samples was 20.50, 25.00 and 30.00% respectively. The potassium (K) compositions of raw unfermented blends were 259.00, 290.00, 270.50 and 321.50% respectively while that of fermented samples was 210.50, 221.50, 163.50 and 163.50% respectively. In addition, the lead (Pb) compositions of raw unfermented blends were 0.10, 0.12, 0.14 and 0.19% respectively while that of fermented samples were all 0.00%. FAO (2001) documented that minerals such as potassium (K), sodium (Na) and magnesium (Mg) are low in cereals but the addition of legumes such as cowpea can improve these mineral contents. Potassium serves as an intracellular cation that binds to protein and sodium and therefore influences osmotic pressure and normal...
pH equilibrium of the body (Oyarekua, 2010). Sodium is a major cation of body fluid cells and the values obtained in this study falls within the recommended potassium/sodium values required for complimentary food formulations for ages 6 to 23 months old. Magnesium is needful for the normal functioning of nerve and muscle cells, maintains a healthy immune system and helps to make the bone strong. However, 0% values were obtained for all the sample blends which implies that these formulations can be consumed without causing any adverse effects that accompanies the consumption of lead contaminated foods such as abdominal pain, seizure, cancer or even death.

Anti-nutrient composition

Tannin contents of unfermented and fermented sorghum-cowpea blends ranged from 0.865 to 0.99 mg/100 and 0.22 to 0.7 mg/100 respectively. Tannin content was highest in fermented cowpea (0.78 mg/100) compared to tannin content in fermented sorghum. Cowpea-sorghum (7:3) and cowpea-sorghum (8:2) blends (0.22, 0.43 and 0.58 mg/100 respectively). Saponin content in raw unfermented cowpea and sorghum samples were not significantly (P<0.05) different giving a yield of 6.63 and 7.08 mg/100 respectively (Table 3). However, values for fermented cowpea, sorghum, cowpea-sorghum (7:3) and sorghum-cowpea (8:2) were 2.68, 1.76, 6.17 and 3.37 mg/100 respectively. The values were significantly different at P<0.05. Oxalate content of unfermented sample blends ranged from 1.16 to 2.93 mg/100 which were significantly different at P<0.05. However, the phytate values of fermented cowpea, sorghum, cowpea-sorghum (7:3) and sorghum-cowpea (8:2) are 1.30, 1.04, 1.72 and 2.93 mg/100 respectively which are significantly different at P<0.05. This report does not agree with the findings of Ojokoh et al. (2014) who reported lower phytate values of 0.59 to 0.93 mg/100 for unfermented breadfruit-cowpea blends and recorded values that ranged between 0.24 to 0.58 mg/100 for fermented sample blends. Ariahu et al. (1999) documented similar phytate values of 1.76 and 1.17 mg/100 for nongerminated-nonfermented soy breadfruit seeds and nongerminated-fermented soy breadfruit seeds blends. Onweluzo and Nnamuchi (2009) also reported high phytate values of 143.3, 125 and 80.13 mg/100 for parboiled, boiled and African fermented breadfruit flour. Ojokoh et al. (2013) has previously reported that the fermenting lactic acid bacteria possesses phytase enzyme that breaks down phytate.

Data are presented as Mean±S.E (n=3). Values with the same superscript letter(s) along the same column are not significantly different (P<0.05).

Table 3. Anti-nutrients composition of sorghum and cowpea blend samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Tannin (mg/g)</th>
<th>Saponin (mg/g)</th>
<th>Oxalate (mg/g)</th>
<th>Phytate (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Raw</td>
<td>Fermented</td>
<td>Raw</td>
<td>Fermented</td>
</tr>
<tr>
<td>Cowpea</td>
<td>0.94±0.01a</td>
<td>0.78±0.02a</td>
<td>6.63±0.09a</td>
<td>2.68±0.03a</td>
</tr>
<tr>
<td>Sorghum</td>
<td>0.86±0.01a</td>
<td>0.22±0.01a</td>
<td>7.08±0.05a</td>
<td>1.76±0.1a</td>
</tr>
<tr>
<td>Cowpea:sorghum (7:3)</td>
<td>0.99±0.02b</td>
<td>0.43±0.03b</td>
<td>13.08±0.5c</td>
<td>6.17±0.3c</td>
</tr>
<tr>
<td>Sorghum: cowpea (8:2)</td>
<td>0.98±0.01bc</td>
<td>0.58±0.02c</td>
<td>9.02±0.22b</td>
<td>3.37±0.02d</td>
</tr>
</tbody>
</table>

Sorghum: cowpea (8:2)  are 1.30, 1.04, 1.72 and 2.93 mg/100 for parboiled, boiled and African fermented breadfruit flour. Ojokoh et al. (1999) during the fermentation of nongerminated and germinated soy-breadfruit blends. High acidity in fermented food products confers microbial stability on the food which helps to reduce the incidence of diarrhea among consumers.

Physico-chemical properties

pH and titratable acidity

The pH of fermenting medium decrease with increase in titratable acidity of the fermented blend samples. The variation in the pH of sample blends may be due to variations in the composition of sample blends supplementation (Figure 1). However, increase in titratable acidity could be attributed to the dominance of the environment by lactic acid bacteria which utilizes the fermentable sugars leading to the acidification of the fermenting medium (Figure 2). Similar decrease in pH and increase in titratable acidity (TTA) had earlier been reported by Ojokoh et al. (2013) during the spontaneous fermentation of breadfruit-cowpea blends, Ariahu et al. (1999) during the fermentation of nongerminated and germinated soy-breadfruit blends. High acidity in fermented food products confers microbial stability on the food which helps to reduce the incidence of diarrhea among consumers.

Microbial characteristics

Table 4 shows that a total of eight (8) bacteria was isolated and identified in the sample blends. Figures 3 to 5 presents the changes in bacterial, fungal and lactic acid loads during the fermentation processes. S. aureus and B. cereus were isolated from the raw sample at the early stage of fermentation of the blends followed by a gradual disappearance towards the end of the fermentation process. llango and Antony (2014) reported similar
Table 4. Biochemical characteristics of all bacterial isolate during fermentation of sorghum and cowpea blend samples.

<table>
<thead>
<tr>
<th>Tests</th>
<th>IS01</th>
<th>IS02</th>
<th>IS03</th>
<th>IS04</th>
<th>IS05</th>
<th>IS06</th>
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<th>IS08</th>
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<tr>
<td>Shape</td>
<td>Cocci</td>
<td>Rod</td>
<td>Rod</td>
<td>Rod</td>
<td>Rod</td>
<td>Rod</td>
<td>Rod</td>
<td>Cocci</td>
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<td>Motility</td>
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<td>+</td>
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<td>-</td>
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<tr>
<td>Spore formation</td>
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<td>Lactose</td>
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</tr>
<tr>
<td>Sucrose</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Galactose</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Suspected</td>
<td>Staphylococcus aureus</td>
<td>Bacillus cereus</td>
<td>B. polymyxa</td>
<td>B. licheniformis</td>
<td>Lactobacillus fermentum</td>
<td>Lactobacillus acidophilus</td>
<td>Lactobacillus plantarum</td>
<td>Streptomyces lactis</td>
</tr>
</tbody>
</table>

+= Positive; -= negative.

Figure 1. pH variation during fermentation of sorghum and cowpea blend samples.

findings during the fermentation of “koozh”, an Indian fermented millet beverage. This implies that these organisms are microbial flora of the raw samples or might have been introduced as a result of inadequate precautionary measures during the processing such as the utensils, water, the environment or even the
Figure 2. Total titratable acidity variation during fermentation of sorghum and cowpea blend samples.

Figure 3. Changes in bacterial load during fermentation of sorghum and cowpea blend samples.
Figure 4. Changes in fungal load during fermentation of sorghum and cowpea blend samples.

Figure 5. Changes in lactic acid bacterial load during fermentation of sorghum and cowpea blend samples.
B. polymyxa and B. licheniformis, L. fermentum, L. acidophilus, L. plantarum and Streptococcus lactis were isolated towards the end of the fermentation process. Several studies have documented that fermenting cereals helps to alter pH levels which do not favor the growth of pathogenic microorganisms due to the production of antimicrobial compounds such as succinic acid, acetic acid, hydrogen peroxide produced by lactic acid bacteria during the fermentation process (Steinkraus, 2002; Hernandez-Ledesma et al., 2004; Odumodu and Inyang, 2006; Ojokoh et al., 2014; Oliveira et al., 2014).

Conclusion

The findings obtained from this study revealed that there is a significant increase in protein content of sorghum supplemented with cowpea and a drastic reduction in the anti-nutrient contents of all the fermented sample blends. Lactic acid bacteria were the dominant microorganisms during the fermentation process. Therefore, sorghum fortified with cowpea, fermented for 72 h can be recommended for improving the quality of the protein quality of sorghum. In addition, this food blend may be recommended as desirable for solving the problem of protein deficiency among the populace especially infants in developing countries.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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