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# 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. acidiophilus*, *L. plantarum* *Streptococcus lactis*, *Aspergillusf lavus, A. 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. Unsuplemented 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, comprises of 70-80 amylopectin 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 (Philips et al., 2003). According to FAOSTAT (2015), Nigeria is the World’s largest producer pf 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 reffinose 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

Model VGB 210 System (2008) edition 6 (AOAC, 2012).

**Anti-nutrient contents**

***Tannin***

0.2 g of finely ground sample was weighed into a 50 ml sample bottle, 10 ml of 70% aqueous acetone was added to it and mixed thoroughly. The bottles were kept in ice bath shaker and shaken for 2 h at 30°C. Each solution was then centrifuged and the supernatant stored in ice. 0.2 ml of the solution was pipetted into a test tube and 0.8 ml of distilled water was added. Standard tannin acid solutions were prepared from a 0.5 mg/ml of the stock and the solution made up to 1 ml with distilled water. 0.5 ml of Folin Ciocaeteau reagent was added to both sample bottles and standardized by pipetting 2.5 ml of 20% $Na_2CO_3$. The bottles were vortexed and incubated for 40 min at room temperature after which its absorbance was read at 725 nm using AJ- IC03 spectrophotometer against a reagent blank concentration of the same solution from a standard tannic acid curve that was prepared (AOAC, 2012).

Tannin acid 1 ml extract = $\frac{R \, x \, 100}{Ml \, of \, sample \, used}$

Where, R = result read from the standard curve.

***Oxalate***

One gram sample was weighed into 1000 ml conical flask. 0.75 M $H_2SO_4$ was added and stirred intermittently with a magnetic stirrer for 1 h. The mixture was filtered using Whatman No. 1 filter paper. A 25 ml of sample filtrate (extract) was collected and titrated hot (80-90°C) against 0.1 $MKMnO_4$ solution to the point when pink colour appeared that persisted for at least 30 seconds (AOAC, 2012)

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***Phytate***

4 g of sample was soaked in 100 ml of 2% HCl for 3 h and filtered using Whatman No. 1 filter paper. 25 ml of the filtrate was placed in 100 ml conical flask and 5 $cm^3$ of 0.03% of ammonium thiocynate solution ($NH_4SCN$) was added as an indicator. 50 ml of distilled water was added to the solution and titrated against 0.00566 g per milliter of standard iron (iii) chloride solution which contains 0.00195 g of iron per milliliter until a brownish yellow colouration appears and lasted up to 5 min. Phytate content in mg/100 g was calculated (AOAC, 2012).

Iron equivalent = litre value x 1.95
Phytic acid = litre value x 1.95 x 1.19 x 3.55 mg/phytic acid.

***Saponin***

0.5 g of sample was weighed into a 20 ml test tube and 10 ml of 80% ethanol was added. The mixture was shaken on a shaker for 5 h to ensure uniform mixing and filtered through a Whatman No. 1 filter paper into a 100 ml beaker. 20 ml of 40% saturated solution of Magnesium carbonate added was added to the filtrate. The mixture was saturated with $MgCO_3$ and filtered again through a Whatman No 1 filter paper to obtain a clear colourless solution. 1 ml of the colourless solution was pipetted into 50 ml volumetric flask and 2 ml of 5% FeCl3 solution was added. The mixture was allowed to stand 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% FeCl solution. The absorbances of the sample as well as standard saponin solutions were read after colour development on a Spectronic 2lD 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).

**Statistical analyses**

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

## 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%

**Table 1.** Proximate composition of fermented and unfermented sorghum and cowpea flour blend samples.

| Sample | Component (%) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Moisture | | Ash | | C .Protein | | C. Fat | | C. Fibre | | Carbohydrate | |
| | Raw | Fermented | Raw | Fermented | Raw | Fermented | Raw | Fermented | Raw | Fermented | Raw | Fermented |
| Cowpea | 0.83±0.06$^{a}$ | 42.65±0.5$^{c}$ | 1.89±0.3$^{d}$ | 0.82±0.01$^{c}$ | 22.63±0.57$^{d}$ | 28.61±0.59$^{d}$ | 2.07±0.02$^{d}$ | 0.93±0.01$^{c}$ | 1.09±0.01$^{a}$ | 0.75±0.01$^{a}$ | 72.57±0.43$^{a}$ | 28.18±0.82$^{a}$ |
| Sorghum | 0.75±0.14$^{a}$ | 28.57±0.5$^{a}$ | 1.06±0.04$^{a}$ | 0.57±0.05$^{a}$ | 4.12±0.07$^{a}$ | 7.20±0.01$^{a}$ | 1.11±0.08$^{b}$ | 0.43±0.01$^{a}$ | 4.90±0.04$^{d}$ | 2.05±0.04$^{d}$ | 89.26±0.83$^{c}$ | 61.85±0.14$^{d}$ |
| Cowpea:sorghum (7:3) | 1.07±0.03$^{a}$ | 39.94±0.$^{b}$ | 1.69±0.02$^{c}$ | 0.67±0.03$^{b}$ | 19.80±0.30$^{c}$ | 22.12±0.18$^{c}$ | 1.77±0.05$^{c}$ | 0.63±0.02$^{b}$ | 2.05±0.01$^{b}$ | 0.89±0.01$^{b}$ | 74.52±0.47$^{a}$ | 36.49±0.01$^{b}$ |
| Sorghum: cowpea (8:2) | 1.03±0.07$^{a}$ | 26.96±0.2$^{a}$ | 1.36±0.05$^{b}$ | 0.50±0.01$^{a}$ | 10.07±0.13$^{b}$ | 13.62±0.58$^{b}$ | 0.88±0.04$^{a}$ | 0.41±0.01$^{a}$ | 3.43±0.03$^{c}$ | 1.27±0.03$^{c}$ | 83.75±0.25$^{b}$ | 59.18±0.82$^{c}$ |

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) whereby moisture content of cowpea-sorghum blends decreased with increased fermentation time. Ojokoh et al. (2014) also observed lower moisture content during the fermentation of cowpea-bread fruit blends (10.91-10.77%).

Crude protein content of unfermented sorghum and cowpea samples were 4.12 and 22.63% respectively. Increasing the proportion of cowpea significantly (p≤0 .05) increased the protein content of the fermented sorghum and cowpea blends. The protein content of fermented sorghum and cowpea blends ranged from 13.62 to 22.12% (Table 1). Fermentation significantly (p≤0.05) increased the protein content of sorghum and cowpea blends compared to the raw samples. Ojokoh et al. (2014) reported a similar observation during the fermentation of bread-fruit-cowpea blends in which the protein content ranged from 7.25 to 24.14%.(Sefa-Dedeh et al. 2001) also documented that fortifying cereals with cowpea improves the protein content of the cereal diet. Bello et al. (2018) reported that food products of plant origin capable of providing more than 12% of its calorific value from protein are considered as good source of protein. Increase in protein content may be due to the increased growth and microbial proliferation in the form of single cell protein and the structural proteins that are intergral part of the microbial cell (Tortora et al., 2002; Wakil and Kazeem, 2012). Cowpea-sorghum blend (7:3) had the highest protein content (22.12%). Earlier works have also documented that protein quality is improved in cereal-cowpea blends due to the synergistic combination effect of lysine by cowpea and methionine by cereals (Bressani, 1993; Wakil and Kazeem, 2012; Momanyi et al., 2019).

The total ash contents of the raw unfermented sorghum and cowpea samples were 1.06 to 1.89% respectively. 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%)

**Table 2.** Mineral composition of sorghum and cowpea blend samples.

| Sample | Minerals (mg/100 g) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | K | | Na | | Mg | | Pb | |
| | Raw | Fermented | Raw | Fermented | Raw | Fermented | Raw | Fermented |
| Cowpea | 320.00±2.0$^{a}$ | 287.50±3.5$^{a}$ | 23.50±1.0$^{a}$ | 11.00±1.0$^{a}$ | 259.00±5.0$^{a}$ | 210.50±0.0$^{b}$ | 0.10±0.0 | 0.00±0.0 |
| Sorghum | 448.00±2.0$^{c}$ | 390.50±1.5$^{b}$ | 30.50±1.0$^{b}$ | 20.50±1.0$^{b}$ | 290.00±2.0$^{c}$ | 221.50±1.0$^{c}$ | 0.12±0.0 | 0.00±0.0 |
| Cowpea:sorghum (7:3) | 434.00±1.0$^{b}$ | 403.50±1.5$^{c}$ | 39.50±1.0$^{c}$ | 25.00±1.0$^{c}$ | 270.50±1.0$^{b}$ | 163.50±1.0$^{a}$ | 0.14±0.0 | 0.00±0.0 |
| Sorghum: cowpea (8:2) | 444.00±2.0$^{c}$ | 395.50±3.5$^{bc}$ | 40.00±2.0$^{c}$ | 30.00±1.0$^{d}$ | 321.50±1.0$^{d}$ | 219.00±1.0$^{c}$ | 0.19±0.0 | 0.00±0.0 |

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).

significantly (p≤0.05). 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 fibre 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 fibre content of fermented sample blends were significantly different at (p≤0 .05). Fermented sorghum and sorghum-cowpea blends (8:2) had a higher fibre 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.

Carbohydrate content of unfermented sorghum-cowpea blends ranged from 74.52-83.75% compared to 89.26% for sorghum and 72.57% for cowpea. Increased proportion of sorghum contributed to a significant (p≤0 .05) increase in carbohydrate content. Fermentation significantly decreased (p≤0 .05) the carbohydrate content of fermented cowpea and 7:3 cowpea-sorghum blends. However, fermented sorghum had the highest carbohydrate content (61.85%). The increase in carbohydrate content with increasing sorghum proportion could be attributed to the high carbohydrate (starch) content of sorghum (Table 1). Ariahu et al. (1999) had earlier documented carbohydrate values of 62.6% and 61.2% for non-germinated and non-fermented soy-breadfruit formulation blends. Low carbohydrate content of fermented cowpea and cowpea-sorghum blends (7:3) (28.18% and 36.49%) respectively could be attributed the low carbohydrate content of cowpea compared to sorghum, utilization of fermentable sugars by the fermenting microorganisms and other metabolic activities (Ojokoh et al., 2013, 2014).

## 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 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 219.00% 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

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

| Sample | Anti-nutrients (mg/g) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Tannin | | Saponin | | Oxalate | | Phytate | |
| | Raw | Fermented | Raw | Fermented | Raw | Fermented | Raw | Fermented |
| Cowpea | 0.94±0.01$^{b}$ | 0.78±0.02$^{d}$ | 6.63±0.09$^{a}$ | 2.68±0.0$^{b}$ | 2.42±0.01$^{c}$ | 1.30±0.04$^{d}$ | 19.73±0.0$^{a}$ | 13.16±0.01$^{a}$ |
| Sorghum | 0.86±0.01$^{a}$ | 0.22±0.01$^{a}$ | 7.08±0.05$^{a}$ | 1.76±0.1$^{a}$ | 1.16±0.01$^{a}$ | 1.04±0.01$^{c}$ | 19.37±0.1$^{a}$ | 17.28±0.02$^{d}$ |
| Cowpea:sorghum (7:3) | 0.99±0.02$^{c}$ | 0.43±0.03$^{b}$ | 13.08±0.5$^{c}$ | 6.17±0.3$^{c}$ | 1.72±0.02$^{b}$ | 0.71±0.01$^{b}$ | 25.88±0.3$^{b}$ | 13.98±0.02$^{b}$ |
| Sorghum: cowpea (8:2) | 0.98±0.01$^{bc}$ | 0.58±0.02$^{c}$ | 9.02±0.22$^{b}$ | 3.37±0.0$^{b}$ | 2.93±0.03$^{d}$ | 0.44±0.01$^{a}$ | 29.66±0.8$^{c}$ | 15.63±0.02$^{c}$ |

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

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 equired 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 in 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 were 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 of143.3, 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.

## 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. Ilango and Antony (2014) reported similar

**Table 4**. Biochemical characteristics of all bacterial isolate during fermentation of sorghum and cowpea blend samples.

| Tests | IS01 | IS02 | IS03 | IS04 | IS05 | IS06 | IS07 | IS08 |
|---|---|---|---|---|---|---|---|---|
| Gram rxn | + | + | + | + | + | + | + | + |
| Shape | Cocci | Rod | Rod | Rod | Rod | Rod | Rod | Cocci |
| Motility | - | + | + | + | - | - | - | - |
| Spore formation | - | + | + | + | - | - | - | - |
| Citrate | - | + | + | + | - | - | + | - |
| Catalase | + | + | - | - | - | - | - | - |
| Coagulase | + | - | - | + | - | - | - | - |
| MR/VP | - | - | + | - | - | + | + | - |
| Glucose | + | + | + | + | - | + | - | - |
| Lactose | + | + | + | + | + | + | + | - |
| Mannitol | + | + | + | - | + | - | - | - |
| Sucrose | - | - | + | + | - | - | - | - |
| Galactose | - | + | + | + | - | - | - | - |
| Suspected organisms | *Staphylococcus aureus* | *Bacillus cereus* | *B. polymyxa* | *B. licheniformis* | *Lactobacillus fermentum* | *Lactobacillus acidiophilus* | *Lactobacillus plantarum* | *Streptomyces lactis* |

+=Positive; -=negative.

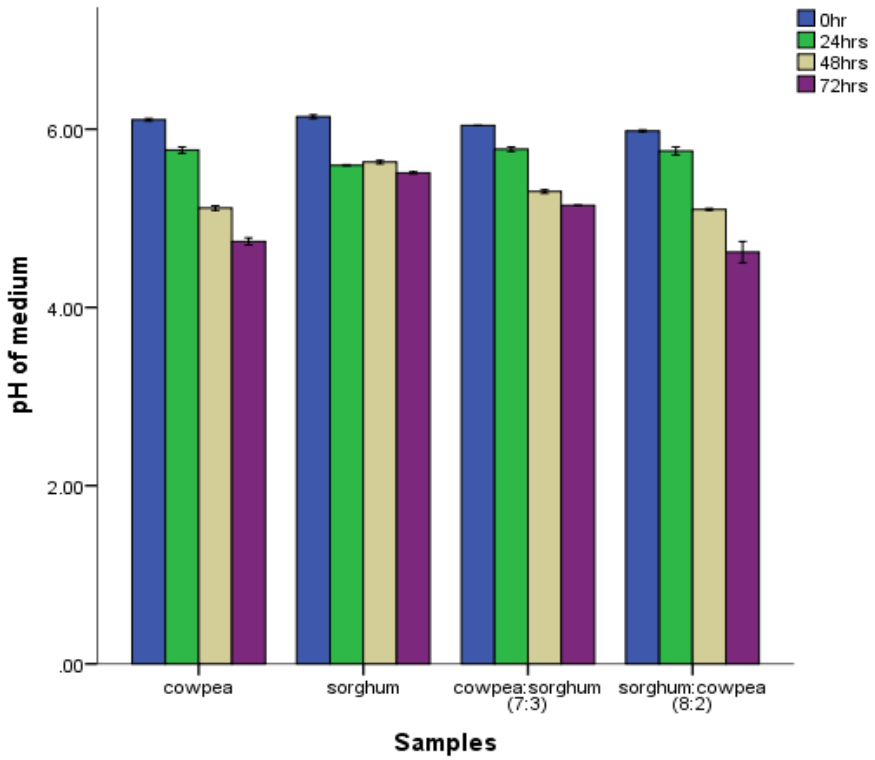


**Figure 1.** pH variation during fermentation of sorghum and cowpea blend samples.

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

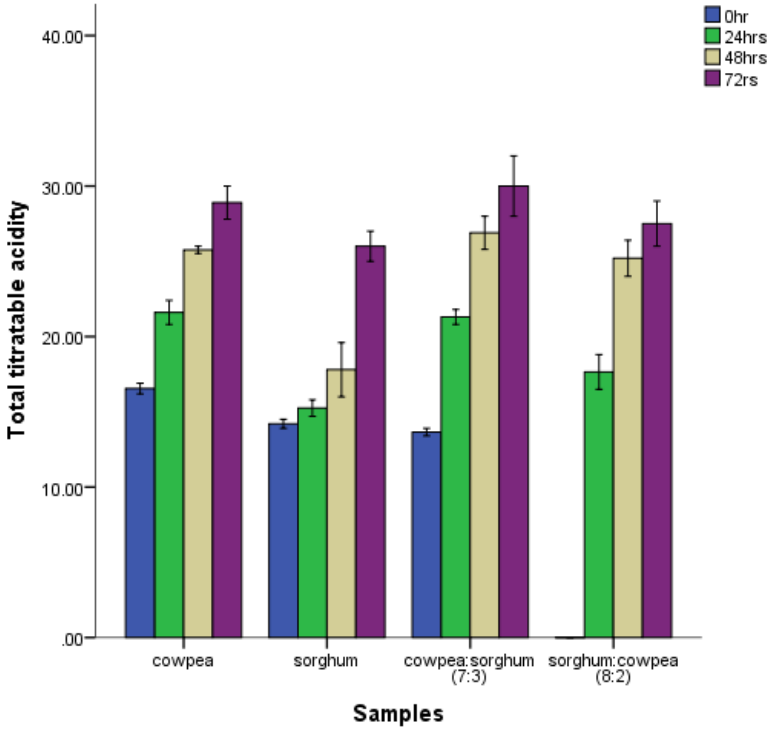


**Figure 2.** Total titratable acidity variation during fermentation of sorghum and cowpea blend samples.

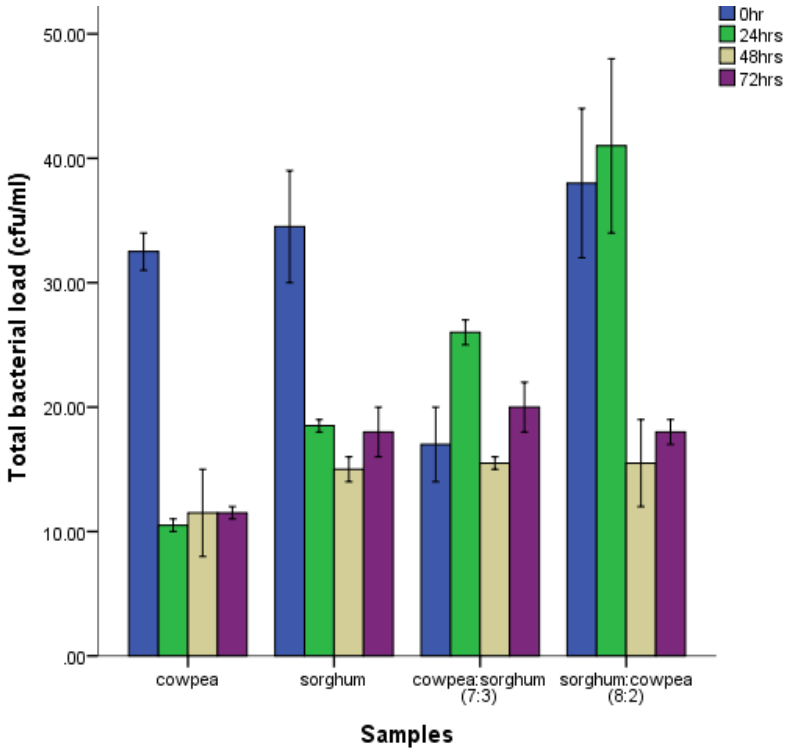


**Figure 3.** Changes in bacterial load during fermentation of sorghum and cowpea blend samples.

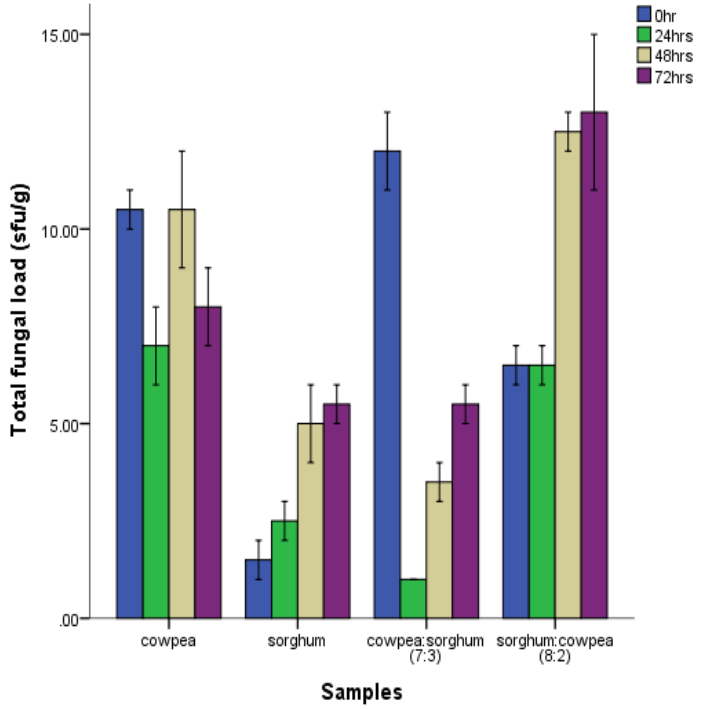


**Figure 4**. Changes in fungal load during fermentation of sorghum and cowpea blend samples.

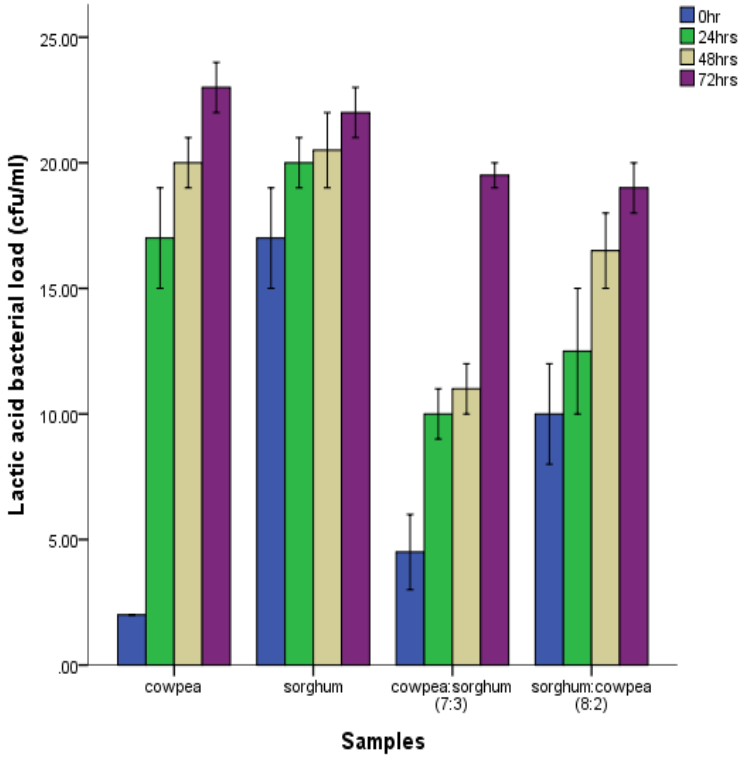


**Figure 5.** Changes in lactic acid bacterial load during fermentation of sorghum and cowpea blend samples.

producers.

*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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