British Microbiology Research Journal
10(6): 1-14, 2015, Article no.BMRJ.19260
ISSN: 2231-0886, NLM ID: 101608140

SCIENCEDOMAIN *international*
www.sciencedomain.org

Effect of Fermentation on Proximate Composition and Microbiological Changes of Sorghum and Pumpkin Blend

Anthony O. Ojokoh[1*] and Ojokoh Eromosele[2]

[1]Department of Microbiology, Federal University of Technology, P.M.B. 704, Akure, Ondo State, Nigeria.
[2]Feed Research Institute, Chinese Academy of Agricultural Sciences, No. 12 Zhongguangcun Street, Beijing 100081, P. R. China.

Authors' contributions

This work was carried out in collaboration between both authors. Author AOO designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript and managed literature searches. Author OE managed the analyses of the study and literature searches. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BMRJ/2015/19260
Editor(s):
(1) Sabina Fijan, University of Maribor, Slovenia.
Reviewers:
(1) Klara Kosova, Crop Research Institute, Prague, Czech Republic.
(2) Anonymous, Evangel University Akaeze, Nigeria.
Complete Peer review History: http://sciencedomain.org/review-history/11633

Original Research Article

Received 31st May 2015
Accepted 30th July 2015
Published 29th September 2015

ABSTRACT

Aim: The aim of this study was to investigate the effect of fermentation on proximate composition, titratable acidity and microbiological changes of sorghum and pumpkin blend. The raw materials used were sorghum and pumpkin flour blend which had three combinations: SpA = 100:0, SpB = 70:30, SpC = 60:40.
Methodology: The blends were subjected to natural fermentation for a period of 72 h. The following microorganisms were isolated from the fermentation process; *Staphylococcus aureus, Staphylococcus saprophyticus, Saccharomyces cerevisiae, Lactobacillus casei, Bacillus subtilis*, *Mucor* sp., *Aspergillus niger.* Among the microorganisms isolated*, Aspergillus fumigatus* and *Lactobacillus plantarum* were found to be the most dominant organisms during the fermentation process. The pH value decreased while titratable acidity (TTA) increased in total in all of the

**Corresponding author: E-mail: anthonyojokoh.caas@gmail.com, tonyojokoh@yahoo.com;*

samples analyzed. Results from the proximate analysis shows that there was a marginal increase in crude protein content for each sample and the increase are as follows: 10.820±0.042 to 14.520±0.042, 19.610±0.014 to 24.010±0.014 and 22.255±0.035 to 27.335±0.021%.

Conclusion: Possible means of getting nutritional benefit components in sorghum is by utilizing fermentation method. Fermentation as investigated in this study revealed that protein and fat content increased, while anti-nutrient content decreased. The content of fat and ash increased while that of carbohydrate and crude fibre decreased after fermentation. There was also a significant decrease in anti-nutritional content of flavonoid, phytate and tannin after 72 h of fermentation.

Keywords: Fermentation; anti-nutritional; microorganisms; flavonoid.

1. INTRODUCTION

Sorghum which can also be called *Sorghum bicolor* (L.) is known to have a variety of names like: great millet or guinea corn in West Africa, 'kafir' corn in South Africa, 'dura' in Sudan, 'mtama' in eastern Africa, 'jowar' in India and 'kaoliang' in China [1]. *Sorghum bicolor* is a universal crop used as food in the form of grain, as syrup which is known as ("sorghum molasses") and as fodder used in the production of alcoholic beverages and biofuels [2]. Most varieties are drought and heat-tolerant, especially in arid regions, where the grains serve as one of the staples for poor and people living in rural communities. *Sorghum bicolor* is an important food crop in Africa, Central America, South Asia and is the "fifth most important cereal crop grown in the world". It constitutes a major source of energy [3]. On the other hand, Pumpkin with botanical name *Cucurbita pepo L* is a herbaceous running plant belonging to the *Cucurbitaceae* family. It is one of the natural plants that grow well in Nigeria, a plant grown for its edible pulp fruit. Pumpkin plant is an annual plant with leafy green vegetable; which has a climbing stem of up to 12 m long and fruit with a round fibrous flesh. Pumpkin fruits vary in size, colour, shape and weight. Pumpkins have moderately hard flesh with a thick edible flesh and a central cavity containing seeds. The pulp is cooked and consumed as food in many parts of Africa, which is also used as a medicine [4]. Past studies have shown that the fruits do not only contain nutritional properties but also possess important phyto-compounds which have significant anti-nutritional effects [5]. The seed of Pumpkin contains vitamins like vitamin E in a large variety, which includes the Alpha-tocopherol, gamma-tocopherol, delta-tocopherol, alpha-tocomonoenol and gamma-tocomonoenol forms of vitamin E [5]. Other compounds include Oxalate, Phytate, Saponins, Nitrate and Cyanide [6]. Cereals are more widely used as food in African countries than in the developed world. In fact, cereals account for 77% of the total calorie consumption in African countries [7]. Fermented cereals are important as dietary staples for adults in Africa. Major cereals grown in Africa include sorghum, rice, maize and millet [8]. Aponte et al. [9], reported that employing natural fermentation to blends of chestnut and rye flour increased nutritional quality, thereby leading to a development of new cereal-based product. Sorghum is one of the cereals cultivated in Africa and it is about the largest cultivating crop in the northern Guinea Savannah areas of Nigeria [10]. Sorghum like many other grains could be used in different ways, this includes human consumption and animal feed. Sorghum fibres are used in wallboard, fences, biodegradable packaging materials and solvents. The dried stalk of sorghum is used for cooking as fuel and dye can be extracted from the plant which can be used in making fabrics [11]. Therefore, the aim of this study was to investigate the effect of fermentation on proximate composition (pH, titratable acidity, proximate and anti-nutrient) of sorghum-pumpkin blends and the microbiological changes.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Preparation of samples

Fresh pumpkin and dried sorghum were obtained from a local market in Itoku and Lafenwa area of Abeokuta, South-West, Nigeria. The shells of the pumpkin were broken and opened to remove the seeds. The seeds were dried under the sun and there after milled. Sorghum was sorted to remove dirty particles, allowed to dry and milled into powder form.

2.1.2 Composition of samples

The sorghum-pumpkin blends were composited into ratios 100:0, 70:30 and 60:40. The method adopted was according to [12]. Labelling was done appropriately to avoid mixed up of all samples (SpA, SpB and SpC).

2.1.3 pH and titratable acidity

About 10 g of each sample was weighed and mixed with 100 ml of distilled water. The mixture was allowed to stand for 15 min with intermittent shaking using a magnetic shaker. After which it was filtered using whatman filter paper. The pH of the filtrate was measured using a pH meter (JENWAY 3015 pH meter). The filtrate was then titrated against 0.1M NaOH to a phenolphthalein end-point.

2.1.3.1 Microorganism plate count

The plate count was carried out according to the method adopted by [13]. 1 g of the sample was added to 10 ml of distilled water to make 10% of the stock solution. 10 fold dilution of the sample was made in distilled water from 10^{-1} to 10^{-10} 1 ml of the diluted sample (10^{-7}) was aseptically transferred into a sterile petri dish and 20 ml of molten agar. Nutrient agar and De Man Rogosa Sharpe Agar (MRS) were also added, and the plate was incubated at 37ºC for 24 h. For Fungi cultures, potato dextrose agar was used and incubated at room temperature for 3 – 5 days.

2.1.4 Microbiological composition analysis

The raw and fermented blend samples were subjected to microbiological composition analysis using a method of serial dilution and pour plated in duplicates. The method adopted for microbiological analysis was according to [14-15].

2.1.4.1 Characterization of bacterial Isolates

Bacterial isolates were identified morphologically and biochemically viz: Gram staining, Spore staining, capsule staining, motility, indole, citrate, urease, oxidase, catalase, coagulase, methyl-red and sugar fermentation test methods adopted was according to [14-16].

2.1.4.2 Proximate analysis

Proximate analysis of sample and procedures for ash, crude fibre, fat, moisture and protein determination was performed according to [17].

2.1.4.3 Moisture content

Moisture content was determined by direct oven drying method; the loss in weight after oven-drying was expressed as % moisture content [18]. Clean and dried Petri-dishes were weighed by using a digital weighing balance and their respective weights were recorded (W1). 5 g of the sample was weighed into pre-weighed dried dishes (W2). Dishes containing samples were transferred into the oven at 105ºC for 3 h. After 3 h of drying, the samples were transferred to a desiccator and allowed to cool before weighing. This process was repeated until a constant weight (W3) was recorded as the percentage moisture content.

$$\% \text{ moisture} = \frac{\text{loss in weight due to drying (W2 – W3)}}{\text{Weight of sample taken (W2 – W1)}} \times \frac{100}{1}$$

2.1.4.4 Ash content

1 g of sample was weighed into clean dried pre-weighed crucibles with lid (W1). The organic matter was burnt off using flame (lid removed) until the sample became charred. The crucible was then transferred to a muffle furnace set at 550ºC (lid removed). Ashing continued until a light grey colour of white ash was obtained. The crucible was then allowed to cool in desiccators and weighed (W2).

$$\% \text{ Ash} = \frac{\text{W2 – W1}}{\text{Weight of Sample}} \times \frac{100}{1}$$

2.1.4.5 Determination of fat content by soxhlet extraction method

Clean and dried thimbles were weighed as (W1) and 5g oven dried sample was added and re-weighed as (W2). Round bottom flask was filled with petroleum ether (40 – 60ºC) up to ¾ of the flask. Soxhlet extractor was fixed with a reflux

condenser and adjusted to gain heat source which allows the solvent to boil gently, the sample was placed inside the thimble and inserted into the soxhlet apparatus. Extraction was carried out with petroleum ether at (40 – 60ºC) under reflux. After extraction, the barrel of the extractor was emptied; while the condenser and the thimble were removed. The extract was then placed inside an oven at 100ºC for 1 h and later allowed to cool in the desiccators, when weighed again is was then recorded as (W3).

$$\% \text{ Fat} = \frac{\text{Weight loss of sample (extracted fat) (W2 – W3)}}{\text{Original weight of the sample (W2 – W1)}} \times \frac{100}{1}$$

2.1.5 Determination of protein content

"Kjeldahl nitrogen method" was adopted for the determination of protein content of the sample. 1.0 g of the sample was weighed into a digestion flask. Kjeldahl catalyst (5 selenium tablets) was added to the sample. 20 ml of concentrated H_2SO_4 was added to the sample and then fixed for 8 h in the digestion unit at (450ºC) of the Kjeldahl apparatus in fume cupboard. The digest, which was pure yellowish in colour after cooling changed into a colourless liquid. It was then transferred into a 100 ml volumetric flask with the addition of distilled water to measure up. 20 ml of 4% boric acid solution was pipette into conical flask and a drop of methyl red was added to the flask as indicator. The sample was further diluted with 75 ml of distilled water. 10 ml of the digest was made with the addition of 20 ml NaOH which was about (20%) and distilled. The steam exit of the distillatory was closed and the change in colour of boric acid solution to green was observed with time. The mixture was distilled for 15 min; method employed was according to [19].

2.1.5.1 Determination of fibre content

2g (W1) of the sample was weighed and transferred into a 1 litre conical flask, 200 ml of hot H_2SO_4 approximately (1.25%) was added and heated gently for 30 min. The mixture was filtered through muslin cloth and then rinsed with hot distilled water. The sample was transferred back into the flask and 200 ml of heated NaOH (1.25%) was added and allowed to boil gently for 30 min. It was filtered through muslin cloth and the residue washed thoroughly with hot distilled water. 10% HCl was used to rinse the residue twice followed by a quick rinse with ethanol which was allowed to dry. The residue was transferred into a crucible, and placed inside the dry oven at 105ºC. The residue was removed from the dry oven, placed into desiccators. It was allowed to dry and weighed as (W2). The residue was ashed at 550ºC for 90 min in a muffle furnace, which was allowed to cool and weighed again as (W3).

2.1.6 Anti-nutrient analyses

2.1.6.1 Flavonoid content

10 g of the sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The solution was filtered through a whatman filter paper (125 mm). The filtrate was then transferred into a crucible and evaporated into dryness using an evaporator over a water bath. The residue was weighed until a constant weight was achieved.

2.1.6.2 Tannin content

0.2 g of grinded sample was weighed and transferred into a 50 ml sample bottle. 10 ml of 70% aqueous acetone was added and covered. The bottle was placed inside an ice bath shaker to prevent the acetone from evaporating and shaking was done for 2 h at 30ºC. Each solution was pipetted into test tubes and 0.8 cm^3 of distilled water was added. Standard tannic solutions were prepared from a 0.5 mg/ml stock and the solution was made up to 1 ml with distilled water. 0.5 cm^3 folin ciocalteau reagents were added to both the prepared sample and standard followed by adding 2.5 ml of 20% sodium carbonate (Na_2CO_3). The solutions were then vortexed and allowed to incubate for 40 min at room temperature, its absorbance was read at 725 nm against a reagent blank concentration of the samples and a standard tannic acid curve was constructed [20].

2.1.6.3 Statistical analysis

Experimental results are expressed as means ± standard deviation (SDs) of three replicates and data were subjected to one way analysis of variance (ANOVA) using SPSS version 14.0. Statistical analysis was performed using EXCEL (version 2007, Microsoft, Inc., New York, USA).

3. RESULTS AND DISCUSSION

3.1 Results

Table 1 represent the sample code and sample ratio used during this study. The samples were composited in 100% sorghum, 70% sorghum and 30% pumpkin, 60% sorghum and 40% pumpkin before the fermentation process.

Table 1. The samples with sample code and sample ratio of sorghum and pumpkin flour

S/N	Sample code	Sorghum (g)	Pumpkin (g)
1	SpA	100	0
2	SpB	70	30
3	SpC	60	40

Key: SpA = Sorghum flour (100 g) + Pumpkin flour (0 g); SpB = Sorghum flour (70 g) + Pumpkin flour (30 g); SpC = Sorghum flour (60 g) + Pumpkin flour (40 g)

Fig. 1 represents the result obtained during the pH analysis of sample during fermentation. The pH values decreased with days of fermentation, where 100% sorghum was recorded as the highest pH value of (6.2 at 0 h) while 60% sorghum: 40% pumpkin had the lowest pH of (4.0 at 72 h) of fermentation. The pH value investigation carried out on all samples showed that pH value decreases with days as fermentation progressed which became more acidic. Result obtained is in accordance with the findings of [21] who reported that pH drops while the titratable acidity increases as the fermentation progressed.

In Fig. 2, the total titratable acidity ranged from 0 h to 72 h of fermentation, the value of titratable acidity (g/100 lactic acid) increases with days of fermentation. The increase in titratable acidity could be as a result of dominance of the environment by lactic acid bacteria which degrade carbohydrates resulting to acidification. This is in agreement with the study carried by [22], where it was reported that total titratable acidity values during fermentation increase with days of fermentation as the pH decreases.

The sample with 60% sorghum: 40% pumpkin had the lowest value of total titratable acid TTA (0.028 at 0 h) and the highest (0.616 at 72 h). The plate count obtained from the fermentation of sorghum–pumpkin blend is shown in Table 2a. For 100% concentration of sorghum at 0 h, the microorganisms isolated were the: *Bacillus subtilis, Bacillus megaterium, Staphylococcus aureus, Saccharomyces cerevisiae,* and *Aspergillus niger* with total viable count of 83 $\times 10^6$ cfu/g, lactic acid bacteria 2.0 $\times 10^6$ cfu/g, yeast 2.0 $\times 10^6$ cfu/g and mold 3.0 $\times 10^6$ cfu/g. For 70% sorghum: 30% pumpkin, microorganisms isolated include: *Bacillus subtilis, Bacillus megaterium, Staphylococcus aureus, Lactobacillus plantarum, Aspergillus fumigatus, Saccharomyces cerevisiae* and *Mucor* sp. with total viable count of 53 $\times 10^6$ cfu/g, lactic acid bacteria 4.0 $\times 10^6$ cfu/g, yeast 3.0 $\times 10^6$ cfu/g and mold 6.0 $\times 10^6$ cfu/g. For 60% sorghum: 40% pumpkin, the microorganisms isolated includes: *Bacillus subtilis, Staphylococcus epidermidis, Klebsiella* sp., *Escherichia coli, L. plantarum, Mucor* sp. with total viable count of 30 $\times 10^6$ cfu/g, lactic acid bacteria 6.0 $\times 10^6$ cfu/g, mold 6.0 $\times 10^6$ cfu/g and there was no yeast growth on the plate.

Table 2b illustrates (i) The microorganisms isolated from 100% sorghum fermentation after 24 h which includes: *Bacillus subtilis, Staphylococcus aureus, Staphylococcus saprophyticus, Klebsiella oxytoca, L. plantarum, Aspergillus fumigatus* with total viable count of 123 $\times 10^6$ cfu/g, lactic acid bacteria 8.0 $\times 10^6$ cfu/g, mold 11.0 $\times 10^6$ cfu/g and there was no yeast growth recorded.

(ii) The microorganisms isolated from 70% sorghum and 30% pumpkin fermentation after 24 h includes: *B. subtilis, Staph. aureus, Staph. saprophyticus L. plantarum, Pseudomonas aeruginosa, Aspergillus fumigatus* and *Rhizopus sp*. with total viable count of 119 $\times 10^6$ cfu/g, lactic acid bacteria 4.0 $\times 10^6$ cfu/g, mold 6.0 $\times 10^6$ cfu/g and there was no yeast growth recorded.

(iii) The microorganisms isolated from 60% sorghum and 40% pumpkin fermentation after 24 h includes: are the *B. subtilis, Staph. aureus, Pseudomonas aeruginosa, Lactobacillus plantarium, Lactobacillus casei,* and *Aspergillus niger* with total viable count of 114 $\times 10^6$ cfu/g, lactic acid bacteria 3.0 $\times 10^6$ cfu/g, mold count 3.0 $\times 10^6$ cfu/g and no yeast growth.

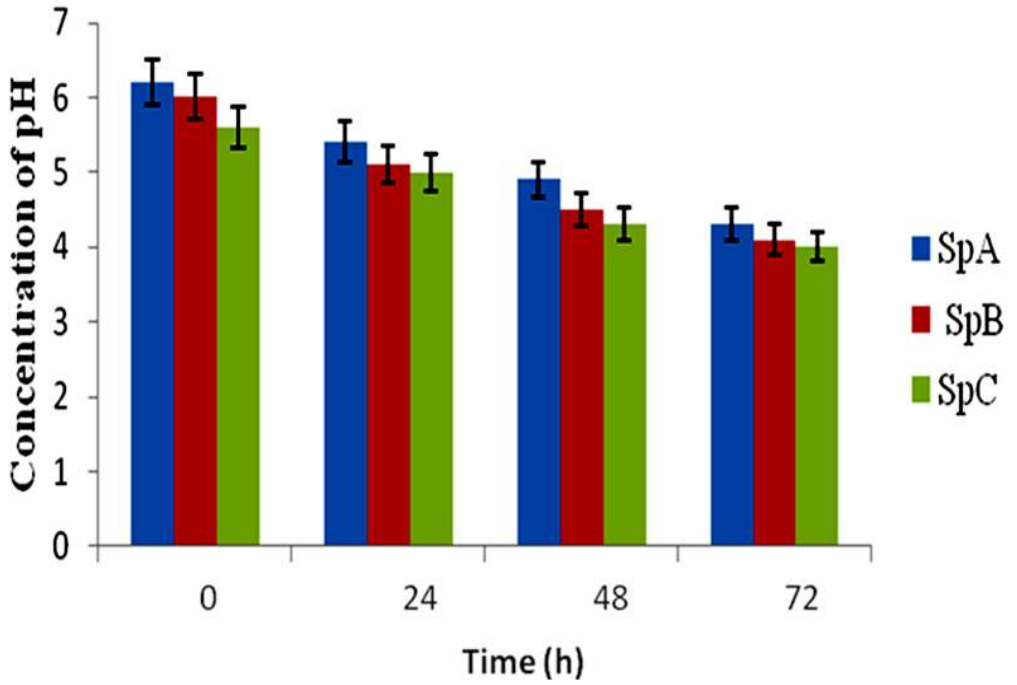

Fig. 1. Total titratable acidity measure during the pH analysis of sample fermentation, where it lasted for 72 h

SpA = 100% Sorghum; SpB = 70% Sorghum + 30% Pumpkin; SpC = 60% Sorghum + 40% Pumpkin

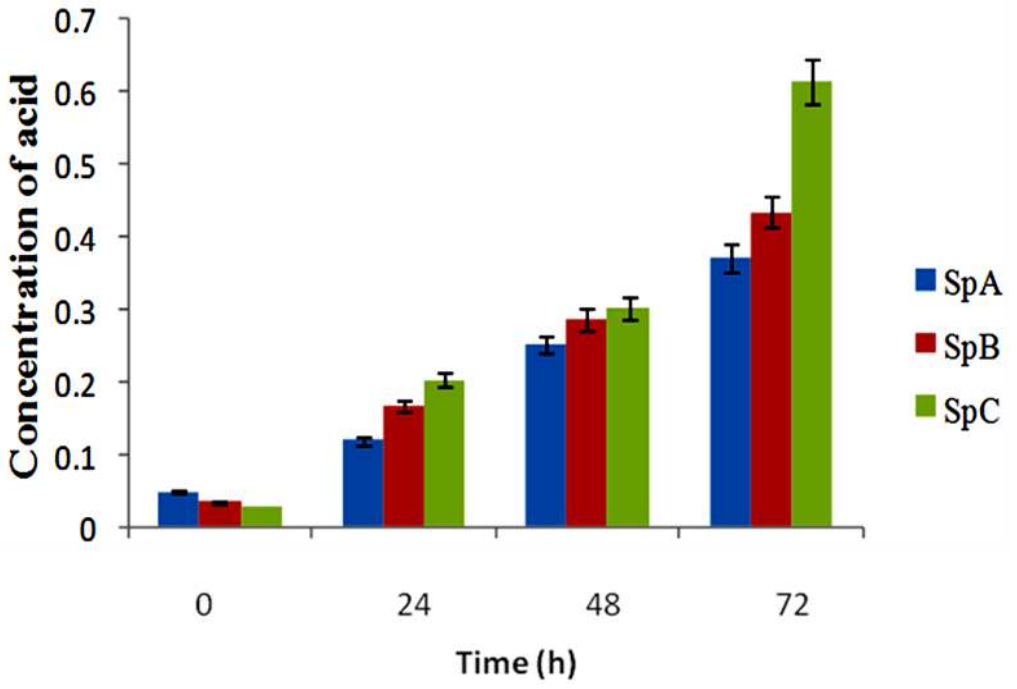

Fig. 2. Total titratable acidity measure during the acid analysis of sample fermentation, where it lasted for 72 h

SpA = 100% Sorghum; SpB = 70% Sorghum + 30% Pumpkin; SpC = 60% Sorghum + 40% Pumpkin

Table (2a). Microorganisms associated with fermentation of sorghum and sorghum-pumpkin blend at 0 hour

Time of fermentation (h)	Concentration (%)	Total viable count (x10^6 CFU/g)	Lactic acid bacteria count (x 10^6 CFU/g)	Yeast count (x10^6 CFU/g)	Mold count (x10^{6}CFU/)	Organisms
0	100	83	2.0	2.0	3.0	*Bacillus subtilis, Bacillus megaterium, Staphylococcus aureus, Saccharomyces cerevisiae, Aspergillus niger, Lactobacillus plantarum*.
0	70	53	4.0	3.0	6.0	*Bacillus subtilis, Bacillus megaterium, Staphylococcus aureus, Lactobacillus plantarum, Aspergillus fumigatus, Saccharomyces cerevisiae, Mucor* sp.
0	60	30	6.0	Nil	8.0	*Bacillus subtilis, Staphylococcus epidermidis, Klebsiella* sp.*, Escherichia coli, Lactobacillus plantarum, Mucor* sp.

Table (2b). Microorganisms associated with fermentation of sorghum and sorghum-pumpkin blend at 24 hours

Time of fermentation (h)	Concentration (%)	Total viable count (x 10^6 CFU/g)	Lactic acid bacteria count (x 10^6 CFU/g)	Yeast count (x 10^6 CFU/g)	Mold count (x 10^6 CFU/g)	Organisms
24	100	123	8.0	Nil	11.0	*Bacillus subtilis, Staphylococcus aureus, Staphylococcus saprophyticus, Klebsiella oxytoca, Lactobacillus plantarum, Aspergillus fumigatus*
24	70	119	4.0	Nil	6.0	*B.subtilis, Staph. aureus, Staph. saprophyticus L. plantarum, Pseudomonas aeruginosa, A. fumigatus, Rhizopus* sp.
24	60	114	3.0	Nil	3.0	*B. subtilis, Staph. aureus, Pseudomonas aeruginosa, L. plantarium, L. casei, A. niger*

The plate count obtained from the fermentation of sorghum–pumpkin blend after 48 h is shown in Table 2c. Result obtained include: (i) The microorganisms isolated from 100% sorghum fermentation after 48 h are the *Bacillus subtilis, Enterobacter sp., Lactobacillus plantarum, Aspergillus niger, Saccharomyces cerevisiae* with total viable count of 212 $\times 10^6$ cfu/g, lactic acid bacteria 5 $\times 10^6$ cfu/g, yeast 4.0 $\times 10^6$ cfu/g and mold 4.0 $\times 10^6$ cfu/g.

(ii) For the microorganisms isolated from 70% sorghum and 30% pumpkin fermentation after 48 h include: *Bacillus subtilis, Pseudomonas aeruginosa, Lactobacillus plantarum, Aspergillus fumigatus, Saccharomyces cerevisiae* with total viable count of 186 $\times 10^6$ cfu/g, lactic acid bacteria 3.0 $\times 10^6$ cfu/g, yeast count 3.0 $\times 10^6$ cfu/g and mold count 3.0 $\times 10^6$ cfu/g.

(iii) The microorganisms isolated from 60% sorghum and 40% pumpkin fermentation after 48 h includes: *Bacillus subtilis, Mucor* sp*., Lactobacillus plantarum, Saccharomyces cerevisiae, Aspergillus niger* with total viable count of 125 $\times 10^6$ cfu/g, lactic acid bacteria 2.0 $\times 10^6$ cfu/g, yeast 2.0 $\times 10^6$ cfu/g) and mold 7.0 $\times 10^6$ cfu/g.

Table 2d shows (i) Microorganisms isolated from 100% sorghum fermentation after 72 h. The following microorganisms were obtained: *Bacillus subtilis, Bacillus megaterium, Lactobacillus plantarum, Aspergillus fumigatus* and *Saccharomyces cerevisiae* with total viable count of 250 $\times 10^6$ cfu/g, lactic acid bacteria 3.0 $\times 10^6$ cfu/g, yeast 1.0 $\times 10^6$ cfu/g and mold count 4.0 $\times 10^6$ cfu/g.

(ii) Microorganisms isolated from 70% sorghum and 30% pumpkin fermentation after 72 h are the *Bacillus subtilis, Pseudomonas aeruginosa, Lactobacillus plantarum, Lactobacillus casei, Aspergillus niger* with total viable count of 205 $\times 10^6$ cfu/g, lactic acid bacteria count 2.0 $\times 10^6$ cfu/g, mold 2.0 $\times 10^6$ cfu/g and there was no yeast growth recorded.

(iii) The microorganisms isolated from 60% sorghum and 40% pumpkin fermentation after 72 h includes: *Bacillus subtilis, Lactobacillus casei, Lactobacillus Plantarum, Aspergillus fumigatus* with total viable count of 198 $\times 10^6$ cfu/g, lactic acid bacteria 1.0 $\times 10^6$ cfu/g while no growth was recorded for yeast and mold after the fermentation process. The microorganisms isolated from the fermentation of Sorghum-pumpkin blend are Bacteria - *Escherichia coli, Klebsiella* sp., *Enterobacter* sp., *Pseudomonas* sp., *Staph. Aureus, Bacillus subtilis, Bacillus megaterium, Lactobacillus planetarium, Lactobacillus casei, Staph. Saprophyticus*, Fungi - *Mucor* sp., *Aspergillus niger*, *Aspergillus fumigatus*, and Yeast – Saccharomyces.

The change in proximate composition obtained from the fermentation of sorghum–pumpkin blend is shown in Table 3. Data shown in Tables 3 and 4 were obtained after 72 h of fermentation, which records the longest fermentation period investigated during the study. The values of moisture content range from 14.810±0.014 to 6.630±0.014 with 100% sorghum (fermented) having the highest moisture content of 14.810±0.014 and 70% sorghum: 30% pumpkin (unfermented) had 6.630±0.014 of moisture content. The values of ash range from 5.550±0.021 to 4.010±0.014 with 60% sorghum: 40% pumpkin (fermented) having the highest ash content of 5.550±0.021 and 100% sorghum (unfermented) having 4.010±0.014. The highest value of fat content obtained was from the mixture of fermented 60% sorghum and 40% pumpkin which is 24.045±0.007 and 100% sorghum (unfermented) having the lowest fat content of 3.565±0.021. Protein content ranged from 10.820±0.042 to 27.335±0.021, where 100% sorghum (unfermented) had the lowest content, 60% sorghum and 40% pumpkin (fermented) had the highest content. Crude fibre content was estimated at 1.465±0.049 for 60% sorghum: 40% pumpkin (fermented), and 100% sorghum (fermented) 0.515±0.021 was recorded. Level of carbohydrate decreased from 73.635±0.049 to 61.090±0.049 for (100% sorghum), 47.850±0.014 to 37.205±0.007 (70% sorghum: 30% pumpkin) and from 42.200±0.113 to 29.655±0.021 60% sorghum: 40% pumpkin) after fermentation for all the samples. Table 4 shows the changes in anti-nutrient composition before and after fermentation of sorghum-pumpkin blend. The anti-nutrients composition decreases in each of the sample after fermentation. Flavonoids had the highest value of 7.700±0.141 for 100% (unfermented) sorghum and the lowest value was recorded at 1.400±0.283 for 70% sorghum: 30% pumpkin (fermented). Phytate had the highest value of 11.945±0.587 in the 60% sorghum: 40% pumpkin (unfermented) concentrations while the lowest was recorded at 4.120±0.000 for 70% sorghum: 30% pumpkin (fermented).

Table (2c). The fermentation of sorghum–pumpkin blend after 48 hours

Time of fermentation (hours)	Concentration (%)	Total viable count ($x10^6$ CFU/g)	Lactic acid bacteria count ($x10^6$ CFU/g)	Yeast count ($x10^6$ CFU/g)	Mold count ($x10^6$ CFU/g)	Organisms
48	100	212	5.0	4.0	4.0	*B. subtilis, Enterobacter sp., L. plantarium, A. niger, Saccharomyces cerevisiae.*
48	70	186	3.0	3.0	3.0	*B. subtilis, P. aeruginosa, L. plantarium, A. fumigatus, S. cerevisiae.*
48	60	125	2.0	2.0	7.0	*B. subtilis, Mucor sp., S. cerevisiae, A. niger.*

Table (2d). Microorganisms associated with fermentation of sorghum and sorghum-pumpkin blend at 72 hours

Time of fermentation (hours)	Concentration (%)	Total viable count ($x\ 10^6$ CFU/g)	lactic acid bacteria count ($x\ 10^6$ CFU/g)	Yeast count ($x\ 10^6$ CFU/g)	Mold count ($x10^6$ CFU/g)	Organisms
72	100	250	3.0	1.0	4.0	*Bacillus subtilis, Bacillus megaterium, Lactobacillus plantarum, Aspergillus fumigatus, Saccharomyces cerevisiae.*
72	70	205	2.0	Nil	2.0	*B. subtilis, P. aeruginosa, L. plantarum, L. casei, A. niger*
72	60	198	1.0	Nil	Nil	*B. subtilis, L. casei, L. plantarum, A. fumigatus*

Table 3. Changes in proximate composition of sorghum-pumpkin blends before and after fermentation (g/100 g) for sample

100% sorghum unfermented	100% sorghum fermented	70%sorghum, 30%pumpkin unfermented	70%sorghum, 30%pumpkin fermented	60%sorghum, 40%pumpkin unfermented	60%sorghum, 40 %pumpkin fermented
Moisture% 6.960±0.014^{c}	14.810±0.014^{f}	6.630±0.014^{a}	10.385±0.021^{d}	6.760±0.014^{b}	12.205±0.021^{e}
ASH% 4.010±0.014^{a}	5.040±0.057^{d}	4.565±0.049^{b}	5.415±0.021^{e}	4.860±0.057^{c}	5.550±0.021^{f}
Fat% 3.565±0.021^{a}	4.025±0.007^{b}	20.010±0.014^{c}	22.015±0.021^{d}	22.510±0.014^{e}	24.045±0.007^{f}
Protein% 10.820±0.042^{a}	14.520±0.042^{b}	19.610±0.014^{c}	24.010±0.014^{e}	22.255±0.035^{d}	27.335±0.021^{f}
Crude fibre%1.010±0.014^{b}	0.515±0.021^{a}	1.335±0.021^{d}	0.970±0.042^{b}	1.465±0.049^{e}	1.210±0.042^{c}
Carbohydrate%73.635±0.049^{f}	61.090±0.049^{e}	47.850±0.014^{d}	37.205±0.007^{b}	42.200±0.113^{c}	29.655±0.021^{a}

Analysis of variance (ANOVA) was used during data evaluation in Table 3. Each value is expressed as mean ± SD. Values with the same superscript alphabets along row are not significantly different

Table 4. Changes in anti-nutrient composition of sorghum-pumpkin blends before and after fermentation (g/100 g) for sample

100% sorghum unfermented	100%sorghum fermented	70%sorghum, 30%pumpkin unfermented	70%sorghum, 30%pumpkin fermented	60%sorghum, 40%pumpkin unfermented	60%sorghum, 40%pumpkin fermented
Flavonoid (%) 7.700±0.141^{d}	4.785±0.021^{c}	4.550±0.212^{c}	1.400±0.283^{a}	2.435±0.049^{b}	2.145±0.205b
Phytate (mg/g) 8.240±0.000^{c}	4.530±0.579^{a}	9.470±0.579^{d}	4.120±0.000^{a}	11.945±0.587^{e}	5.350±0.579^{b}
Tannin (mg/g) 0.301±0.141^{d}	0.132±0.011^{b}	0.238±0.032^{c}	0.009±0.001^{a}	0.114±0.001^{b}	5.350±0.579^{b}

Analysis of variance (ANOVA) was used during data evaluation in Table 4. Each value is expressed as mean ± SD. Values with the same superscript alphabets along row are not significantly different

Tannin had the highest value of 0.301±0.141 in the 100% sorghum (unfermented) and the lowest was recorded at 0.009±0.001 for the (fermented) 70% sorghum: 30% pumpkin.

3.2 Discussion

3.2.1 Production of microorganisms during fermentation

The increase in microorganism's production indicates the presence of essential nutrients that supported the growth of the microorganisms, which indicates that there are possibilities that the microorganisms must have utilized the nutrients, thereby releasing some substances through extracellular activities. Among the microorganisms produced, the *Bacillus subtilis, Lactobacillus* species, *Aspergillus niger, Aspergillus fumigatus and Saccharomyces cerevisiae* were the major microorganisms responsible for the fermentation of the sample. It was observed that high moisture content was recorded for the fermented samples which ranged from 6.960±0.014 to 14.810±0.014, 6.630±0.014 to 10.385±0.021 and from 6.760±0.014 to 12.205±0.021%, when compared to the unfermented sample. The increase in microorganism's production could be as a result of the addition of water to the blends prior to the fermentation process. Protein was observed to have increased in content after fermentation which rose from 10.820±0.042 to 14.520±0.042, 19.610±0.014 to 24.010±0.014 and from 22.255±0.035 to 27.335±0.021% respectively. The reason for the increase in protein content could be as a result of the metabolic activities of the microorganisms which resulted to the release of extracellular enzymes into the samples as reported by other studies carried out by [23].

3.3 Fermentation Process of Sorghum and Pumpkin Blend

Fermentation is a method which can be used to improve food product functionality and protein contents; therefore the results obtained during our study corresponded with the research findings of [24]. Fat increased in its content after the fermentation process of sorghum-pumpkin blends from 3.565±0.021 to 4.025±0.007, 20.010±0.014 to 22.015±0.021 and from 22.510±0.014 to 24.045±0.007%. The results obtained for the increase in fat levels as observed in this study agrees with the findings of [25]. There was an increase in ash content after fermentation of sorghum-pumpkin blends from 4.010±0.014 to 5.040±0.0572.37, 4.565±0.049 to 5.415±0.021 and from 4.860±0.057 to 5.550±0.021%. Since the ash content is a measure of the total amount of minerals present within a food sample, an increase in its level during microbial fermentation could be as a result of incomplete utilization of minerals by fermenting organisms during their metabolism. As part of the observations made during the course of this study, a decrease in crude fibre content was recorded after fermentation of sorghum-pumpkin blends from 1.010±0.014 to 0.515±0.021, 1.335±0.021 to 0.970±0.042% and from 1.465±0.049 to 1.210±0.042%. The reduction in crude fibre could be attributed to enzymatic breakdown of the fibre by the fermenting microorganisms. Although, food containing fibre are known to expand the inner walls of the colon, easing the passage of waste, thus making it an effective anti-constipation, lowering the cholesterol level in the blood and reduce the risk of various cancers. But emphasis has been placed on the importance of keeping fibre intake low in the nutrition of infants and weaning children because high fibre level in weaning diet can lead to irritation of the gut mucosa [26]. Carbohydrate level of all fermented sorghum-pumpkin blend sample decreased from 61.090±0.049, 37.205±0.007 and 29.655±0.021, than those in the raw sample from 73.635±0.049, 47.850±0.014 and 42.200±0.113. The reduction in carbohydrate content was due to the utilization of starch by the microorganisms to glucose which served as carbon source for synthesizing biomass rich in protein. The carbohydrate reduction level result obtained in this study agrees with the study conducted by [27]. They reported that carbohydrate level during fermentation reduced due to the activities of fermenting microbes, which was also observed during this study.

3.3.1 Effect of fermentation on anti-nutritive compounds

Anti-nutrients are compounds which affect the nutritive value of food products such as flavonoid, phytate, and tannin. Before fermentation, the composition of these anti-nutrients content was high in all the samples but after fermentation, the anti-nutrients content were seen to have decreased. Flavonoid content decrease from 7.700±0.141 to 4.785±0.021,

4.550±0.212 to 1.400±0.283 and 2.435±0.049 to 2.145±0.205 after fermentation. Flavonoids in sorghum are derivatives of the monomeric polyphenol flavan-4-ol, which is called anthocyanidins [28]. Anti-nutrients with nutritive value of food products such as Phytates are naturally present in many foods especially cereals and legumes. When above a certain level, phytate reduces the availability of minerals and solubility, functionality and digestibility of proteins [29]. There was significant reduction in phytate level in all of the samples after fermentation from 8.240±0.000 to 4.530±0.579, 9.470±0.579 to 4.120±0.000 and from 11.945±0.587 to 5.350±0.579 mg/100 g were obtained during analysis. The result obtained is in accordance with the findings of [30] Roos et al. (1990) who reported that phytate level drops as fermentation progressed. Tannins are polymers resulting from condensation of flavan-3-ols. Tannins bind to both exogenous and endogenous proteins including enzymes of the digestive tract, affecting the utilization of proteins and iron absorption with high-tannin sorghum [31]. There was significant reduction in tannin level in all of the samples after fermentation. The following values were recorded; 0.301±0.141 to 0.132±0.011 0.238±0.032 to 0.009±0.001 and from 0.114±0.001 to 0.037±0.002 mg/100 g. Some of the anti-nutritional effects of high-tannin sorghum were as a result of low molecular-weight flavonoids which are readily absorbed, inhibiting the metabolic utilization of digested and absorbed foodstuffs [32]. The reduction in anti-nutrient content of all samples indicates the ability of the microorganisms to use them up. The results obtained from this study shows that sorghum-pumpkin blends which contains some naturally occurring toxins /anti-nutrients exist at levels which they occur coupled with naturally fermenting organisms such as *Lactobacillus plantarum, Bacillus subtilis, Lactobacillus casei*, *Aspergilljus niger* and *Aspergillus fumigatus* making their presence of little concern.

4. CONCLUSION

In conclusion, one sure way of getting nutritional benefit substance in sorghum is by fermentation process. Fermentation as studied in this research work proves that protein and fat content increased, while anti-nutrient content decreased. Therefore, fermentation should be recommended as a method for fermenting food in the food industry alongside other food products. Fermented food should be recommended for human consumption for both adult and children since its nutritional value is higher when compared with some raw food.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Purseglove JW. Tropical crops. Longman Limited, London. 1972;519.
2. Mutegi E, Fabrice S, Moses Ben K, Bernard R, Caroline M, Charles M, Joseph K, Heiko P, Santie de V, Kassa S, Pierre T, Maryke L. Ecogeographical distribution of wild, weedy and cultivated Sorghum bicolor (L.) Moench in Kenya: Implications for conservation and crop-to-wild gene flow". Genetic Res and Crop Evolut. 2010; 57(2):243–253.
3. Michodjehoun-Mestres L, Hounhouigan DJ, Dossou J, Mestres C. African J. Biotechnol. 2005;4(6):487-496.
4. Dietmar F. Extract of Pumpkin Seeds Suppresses Stimulated Peripheral Blood Mononuclear Cell *in vitro*. American J. of Immunol. 2005;(1):6-11.
5. Butinar B, Bucar-Miklavcic M, Mariani C. New vitamin E isomers (gamma-tocomonoenol and alpha-tocomonoenol) in seeds, roasted seeds and roasted seed oil from the Slovenian pumpkin variety 'Slovenska golica'. Food Chem. 2011;128:(2):505-512.
6. Omorayi FO, Dilworth L. Anti-nutritional Factors, Zinc, Iron and Calcium in some Carcariggean tuber Crops and Effect of Boiling or Roasting. Nutr and Food Sci. 2007;37(1):8-15.
7. Alias C, Linden G. Food biochemistry. Aspen Publishers Inc. 1999;140-143.
8. Norman FH, Odunfa SA, Cherl-Ho L, Quintero-Ramirez R. Fermented cereals: A global perspective. Food and Agricultural Organization (F.A.O);. 1999. Bulletin No. 38.
9. Aponte M, Boscaino F, Sorrentino A, Coppola R, Masi P, Romano A. Effects of fermentation and rye flour on microstructure and volatile compounds of chestnut flour based sourdoughs. LWT - Food Sci and Technol. 2014;58(2): 387–395.

10. Kolawole OM, Kayode RMO, Akinduyo B. Proximate and Microbial Analysis of Burukutu and Pito Produced in Ilorin, Nigeria. African J. Biotechnol. 2007;6(5): 587-590.
11. Maunder B. Sorghum: The global grain of the future" from National Sorghum Producers. 2006;101.
12. Malleshi JK, Narvhus JA, Sorhaug T. Use of starter cultures of lactic bacteria and yeasts in the preparation of togwa, A Tanzanian fermented food. Dept of food science, Agriculture university of Norway, P.O. box 5036, N-1432 Norway; 1989.
13. Ochei J, Kolhatkar A. Medical laboratory science: Theory and practice book. Tata McGraw Hill Education. 2008;118-127.
14. Bachoon, Dave S, Wendy A. Dustman. Microbiology laboratory manual. Ed. Michael Stranz. Mason, OH: Cengage Learning. Exercise 15, "Normal Flora of the Intestinal Tract" 2008 Print.
15. Ojokoh AO, Daramola MK, and Ochukwa AD. Studies on the microbiological, proximate composition, and anti-nutritional content of fermented groundnut and plantain blends. The pacific J. science and Technol; 2014.
Available:http://www.akamaiuniversity.us/
16. Fawole MO, Oso BA. Laboratory manual of microbiology. Spectrum book Ltd., Ring road, Ibadan. 2004;45-54.
17. AOAC. Official methods of analysis, 14th ed. Association of Official Analytical Chemists Washington DC. 1995;125-126, 132, 877-878.
18. AOAC. Official methods of analysis, (18th Edition). Association of Official Analytical Chemists, Washington, D.C; 2006.
19. AOAC. Official methods of analysis (23th Edition). Association of Official Analytical Chemists, Washington, D.C; 2005.
20. Makkar PSH, Good Child VA. Quantification of tannins: A laboratory manual. International Centre for Agricultural Research in Dry Areas, Aleppo, Syria. 1996;298-307.
21. Mbate CMBK, Narvhus JA, Treimo J, Langsrud T. Isolation, characterization and identification of lactic acid bacteria from bushera: A Ugandan traditional fermented beverage. Inter. J. Food Microbiol. 2008; 80:255-259.
22. Nout AO, Hounhouigan DJ, Dossou J, Mestres C. Physical, chemical and microbiological changes during natural fermentation ‘‘gowe’’, a sprouted or non sprouted sorghum beverage from West – Africa. African J. Biotechnol. 1989;4(6): 487–496.
23. Oboh G, Akindahunsi AA. Biochemical changes in cassava product (flour and garri) subjected to *Saccharomyces cerevisiae* solid media fermentation. Food Chem. 2003;82:599-602.
24. Afoakwa EO, Sefa-Dedeh S, Kluvitse Y, Sakyi-Dawson EO. The influence of fermentation and cowpea fortification on the quality characteristics of maize- based weaning foods. Paper presented at the second international workshop on food-based approaches for healthy nutrition in West Africa: The Role of Food Technologists and Nutritionists, Ouagadougou, Burkina Faso. 2004;23-28.
25. Ojokoh AO. Changes in nutrient and anti-nutrient contents of sweet potato (*Ipomea batatas*) peels subjected to solid substrate fermentation. Bioscience, Biotech, Research Asia. 2005a;3(1):17–20.
26. Bello MO, Farade OS, Adewusi SRA, Olawore NO. Studies of some lesser known nigerian fruits. African J. Biotechnol. 2008;7(1):3972-3979.
27. Odunfa SA, Oyewole. Biochemical changes during production of “ogiri”. A fermented melon (*Citrus vulgaris schard*) product, *Qualitas Plantarum*: Plant Foods for Human Nutri. 1992;32:45-52.
28. Betancur-Ancona D, Gallegos-Tintore S, Ddelgado-Herrera A, Perez- Flores V, Ruelas AC, Luis Chel-Guerrero L. Some Physicochemical and Anti-nutritional Properties of Raw Flours and Protein Isolates from *Mucuna pruriens* (velvet bean) and *Canavalia ensiformis* (jack bean). Inte. J. Food Science and Tech. 2008;43:816-823.
29. Akubo PI, Badifu GO. Chemical composition, functional properties and baking potential of African breadfruit kernel and wheat flour blends. Inter. J. Food Science and Tech. 2004;39:223-229.
30. Roos YH, Young CY. Peanut science and technology, American peanut Research and Education Society, Inc. Yoakum, Texas, USA; 1990.
31. Ojokoh AO. Effect of fermentation on the nutritional qualities of roselle (*Hibiscus sabdariffa Linn*) calyx Ph.D thesis, Federal

University of Technology, Akure, Nigeria. 2005b;170.

32. Perez- Flores V, Ruelas AC, Luis Chel-Guerrero L. Some Physicochemical and Anti-nutritional Properties of Raw Flours and Protein Isolates from *Mucuna pruriens* (velvet bean) and *Canavalia ensiformis* (jack bean). Inter. J. Food Science and Technol. 2008;l43:816-823.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/11633