PROXIMATE COMPOSITION AND ANTINUTRIENT CONTENT OF PUMPKIN *(CUCURBITA PEPO)* AND SORGHUM *(SORGHUM BICOLOR)* FLOUR BLENDS FERMENTED WITH *LACTOBACILLUS PLANTARUM, ASPERGILLUS NIGER* AND *BACILLUS SUBTILIS.*

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ABSTRACT

This study investigated proximate composition, antinutritional contents and physicochemical properties of pumpkin (*Cucurbita pepo*) and sorghum (*Sorghum bicolor*) flour blends fermented with pure strains of *Lactobacillus plantarum, Aspergillus niger* and *Bacillus subtilis*. Proximate composition, hydrogen cyanide and oxalate and phenol contents were determined. pH and titratable acidity were also determined. A significant increase ($p < 0.05$) was observed in the ash, protein and fat contents of the fermented samples especially in the sample fermented with *Aspergillus niger* (6.01%, 21.51% and 21.05%) compared to the unfermented sample(4.01%,10.82% and 3.57%). Significant reductions were observed in carbohydrate content after fermentation. Fermentation process caused a significant decrease ($p < 0.05$) in the hydrogen cyanide, oxalate and phenol contents of the pumpkin-sorghum flour blends. The changes observed in the fermented flour blends agreed with significant decreases recorded for pH and increases in titratable acidity. Fermentation improves the nutritional composition of pumpkin-sorghum flour blends for possible use as complementary foods for infants.

Key words: Pumpkin-sorghum flour blends, fermentation, physico-chemical, anti-nutrient

INTRODUCTION

There is an increased interest in the production of flours from locally available and abundantly grown grains and pulses and fermentation is one of the choice methods employed. Traditional cereal foods play an important role in the diet of the people of Africa particularly in cereal producing zones. Flour from various cereals is one of the main raw materials used in the production of popular food products with high acceptability, good storage characteristics and affordable cost (Akinrele, 1970)

Fermentation is an age-long method of processing cereals and legumes (Siegel and Fawcet, 1978). It modifies some physical characteristics of cereals and legumes, increases the level of some nutrients, digestibility and bioavailability (Ojokoh and Yimin, 2009), decreases levels of antinutrients, increases nutrient density (Nnam, 1999)and imparts some antimicrobial property (Mensah *et al.*, 1990). According to Quinn *et al.* (1975), fermentation of grains and oil seeds results in increased nutritional value and wholesomeness over the starting material and it may also lead to changes in vitamin levels.

Food fermentation, and especially lactic acid fermentation, is an important technology in Africa. The technology is indigenous and is adaptable to the culture of the people. The fermentation process meets the requirements of low-cost, prevention of food spoilage and food-borne diseases with respect to consumers living in a climate which favours the rapid deterioration of food. In addition, fermented foods are of particular importance in ensuring adequate intake of protein and/or calories in the diet (Oyewole and Odunfa, 1989).

Fermentation actually holds promise as a food processing method that can be used to diversify the food uses of some plant foods like sorghum and pumpkin. The traditional processing of these foods therefore needs to be changed or modified to improve their nutritional status. Fortification of popularly consumed staple foods such as cereals with legumes is being exploited in many developing countries. In this process, the protein quality of staple foods is improved through a mutual complementation of their limiting amino acids. Cereal-legume mixtures make a very significant contribution towards the alleviation of protein-energy malnutrition (Plahar *et al.*,1997), and pumpkin is one of the commonly used legumes during cereal-legume fortifications. Pumpkin has, in recent times become popular in the West African sub-region where it is being

cultivated at a steadily increasing rate.

Although, fermentation improves the nutritive value of cowpea, the process is not popular in Nigeria. Fermentation of pumpkin with sorghum could be a useful process of improving the health of vast numbers of infants and children at low cost especially in the rural areas. This might offer a significantly cheap and sustainable food process that will reduce micronutrient deficiency. In this study, the proximate and antinutritional properties of pumpkin and sorghum flour blends that were fermented with pure strains of *Lactobacillus plantarum, Aspergillus niger* and *Bacillus* isolated from spontaneous fermentation of pumpkin and sorghum blends were investigated.

MATERIALS AND METHODS

Source of Samples

The common variety of sorghum in Nigeria (*Sorghum bicolor)* was purchased from a local retail outlet in Oja-Oba market, Akure, Ondo state. Pumpkin (*Cucurbita pepo)* was purchased from local retail outlet in Eleyewo market, Ikole Ekiti,Ekiti State.

Sample Preparation

The seeds of pumpkin were removed with knife, washed, and sun-dried. Thereafter the seed was de- shelled manually using hand, sundried again and ground into powder with a hammer mill. The pumpkin seed flour was packed in airtight polythene bag prior to analysis. Sorghum grains were ground into powder, packed in airtight polythene bag prior to analysis.

Source of Microorganisms

Lactobacillus plantarium, Bacillus subtilis and *Aspergillus niger* used for the fermentation were isolated from previous study on spontaneous fermentation of pumpkin and sorghum.They were subcultured on de Man Rogosa Sharpe (MRS), Nutrient agar (NA) and Potato dextrose agar (PDA) respectively and incubated appropriately. The strains were selected after morphological, phenotypic and molecular characterization as described by Sawitzki *et al* (2007). The isolated strains were preserved in microbial vials.

Formulation of Samples

The sorghum-pumpkin blends were formulated in ratios 100:0, 70:30, and 60:40 (grams) each into three conical flasks and were labelled and coded appropriately to avoid mix up of samples. For ratio 100:0 it was labelled- SPA 1, SPA 2, SPA 3. For ratio 70:30, - SPB 1,SPB 2, SPB 3, while for ratio 60:40 - SPC 1, SPC 2 and SPC 3.

Fermentation

Flours were mixed with 200ml of distilled water and then shaken together thoroughly before autoclaving at 121°C for 15minute. After autoclaving, each sample was allowed to cool. Ten ml of each isolate (*Lactobacillus plantarum, Aspergillus niger, Bacillus subtilis)* was introduced aseptically separately into each sample. Each sample (the flour blends) was fermented in a conical flask for 72 hours at room temperature and physicochemical analysis (pH, total titratable acidity, proximate analysis and anti-nutritional factors analysis) were carried out both on the raw (unfermented) and fermented samples.

Physico-Chemical Properties
pH and Titratable Acidity

The method described by AOAC (2005) was used to determine pH and titratable acidity of the fermenting medium. Samples were taken every 24 hrs during the fermentation period and homogenised according to the procedure described by Fayemi and Ojokoh (2012). Samples were measured using an Orion pH meter (Model 310, Orion Research Inc., Beverly, MA) equipped with glass electrode. The pH meter was calibrated with KOH buffer solutions of pH 7.0 and 4.0 before the measurements. The titratable acidity (TTA) was determined by titrating 20 ml of the homogenised sample against 0.1 M NaOH using phenolphthalein as an indicator. Values obtained were expressed as percent lactic acid. All analyses were carried out in triplicates.

Proximate Composition

The moisture, crude protein (N x 6.25), crude fibre, crude fat and total ash contents of breadfruit-cowpea flour blends (composites) were determined before and after 72 hrs of fermentation using the method described by Association of Official Analytical Chemists' (AOAC, 2005) approved methods 925.10, 920.87,

920.86, 920.39 and 923.03 respectively. Total carbohydrate content of the samples was calculated by difference method (subtracting the percent moisture, crude protein, crude fibre, crude fat, and ash from 100 %)

Antinutrient Contents

Phenol and hydrogen cyanide (HCN) contents were determined by AOAC (2005) method. Oxalate content was measured according to the titrimetric method (AOAC, 2005)

Statistical Analysis

All measurements were carried out in triplicate. Analysis of variance (ANOVA) was conducted on the data at $p < 0.05$ using MINITAB statistical software (Minitab® Release 14.13, Minitab Inc., USA). The least significant difference (LSD) at 95% confidence level was computed to ascertain where differences existed.

RESULTS

The changes in the moisture content of the samples are shown in the Figure1. There was increase in the moisture content of all the samples. The mean moisture content for unfermented sorghum sample was 6.96% and for the fermented samples were 13.02%, 11.73% and 10.93% for *Lactobacillus plantarum, Bacillus subtilis* and *Aspergillus niger fermented samples* respectively. The mean crude protein for fermented samples were 21.51%, 18.51% and 14.52% for samples fermented with *Aspergillus niger, Lactobacillus plantarum* and *Bacillus subtilis* respectively (Figure 2). The mean protein content of unfermented sorghum was (10.82%),

The mean crude fat for fermented samples were 21.05%, 10.05% and 8.05% for *Aspergillus niger, Lactobacillus plantarum* and *Bacillus subtilis* fermented samples compared to that of unfermented sample (3.57%; Figure 3). The mean ash content for fermented samples was 6.01% compared to that of unfermented sample (4.01%; Figure 4). The mean value of crude fibre for fermented samples was 1.52% while that of unfermented sample was (1.10%; Figure5). There was decline in the level of carbohydrates in the samples. The mean for fermented samples were 37.14%, 48.08%, and 62.63% for *Lactobacillus plantarum, Aspergillus niger and Bacillus subtilis* fermented samples while that of unfermented samples was 73.67% (Figure 6).

Figures 7 and 8 present the result of the pH and total titratable acidity after fermentation. The mean pH and titratable acidity for the *Lactobacillus plantarum, Aspergillus niger* and *Bacillus subtilis* fermented samples were 4.2, 4.2 and 4.1 and 0.42, 0.43 and 0.39, respectively.

Observed changes in the levels of anti-nutrient are recorded in Figures 9-11. There was decrease in the hydrogen cyanide of all samples. The mean hydrogen cyanide content of fermented samples was 13.02mg/100g and unfermented 16.21mg/100g. There was decrease in the oxalates content of all samples. The mean of the oxalates content of fermented samples was 0.23mg/100g, while that of unfermented was 1.69mg/100g. The mean value for the phenol content in the fermented samples was 3.58m/100g and that of unfermented samples was 4.49mg/100g.

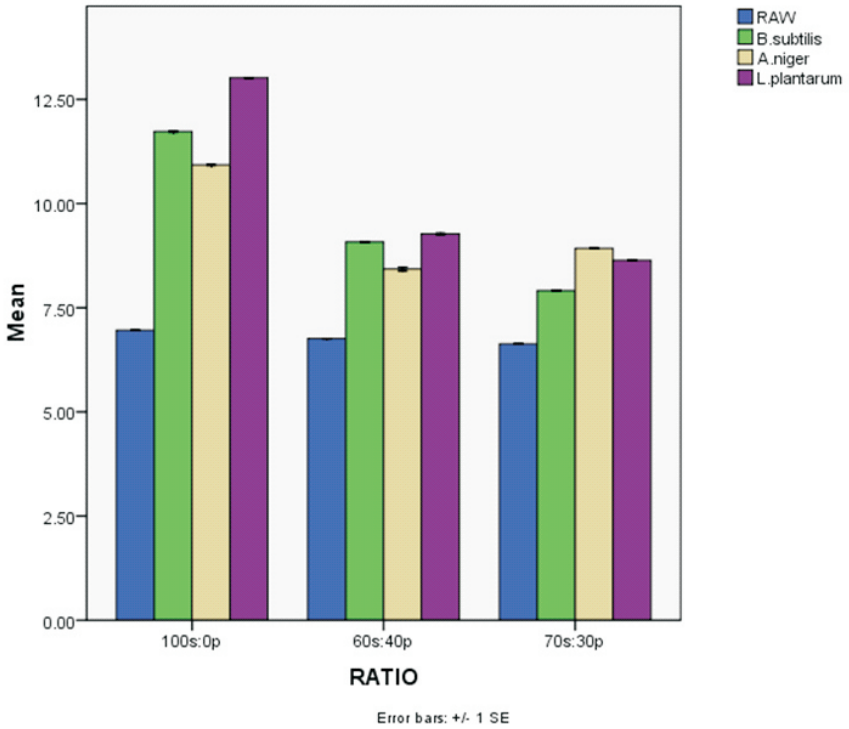

Figure 1: Moisture content (%) of sorghum unfortified and fortified with pumpkin before and after fermentation (72hr)
Bars are presented as Mean ±S.E of replicates (n=3)
Key: s= sorghum, p= pumpkin

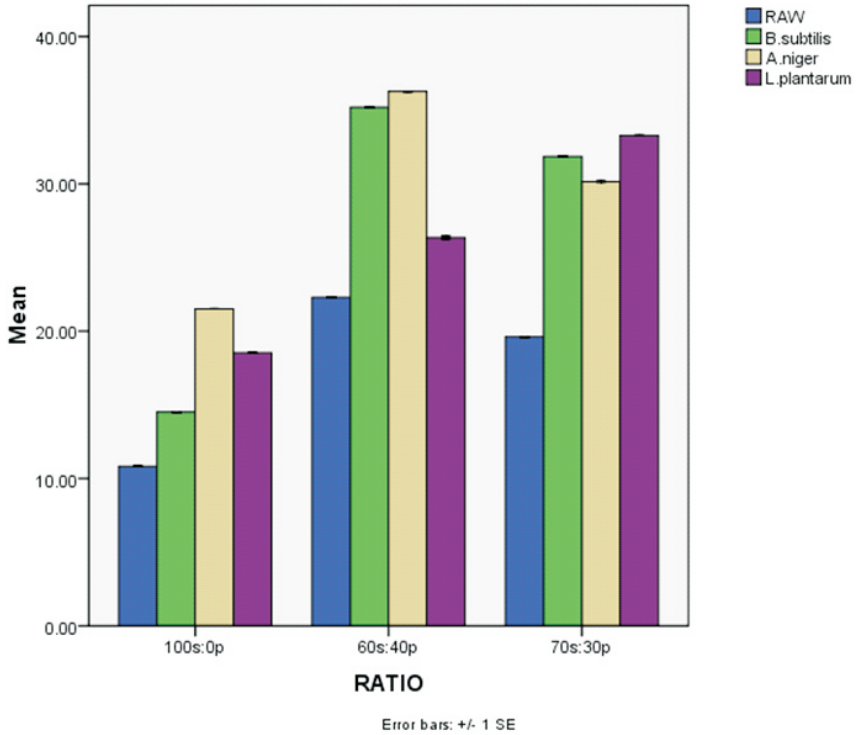

Figure 2: Crude protein (%) of sorghum unfortified and fortified with pumpkin before and after fermentation (72hr)
Bars are presented as Mean ±S.E of replicates (n=3)
Key: s= sorghum, p= pumpkin

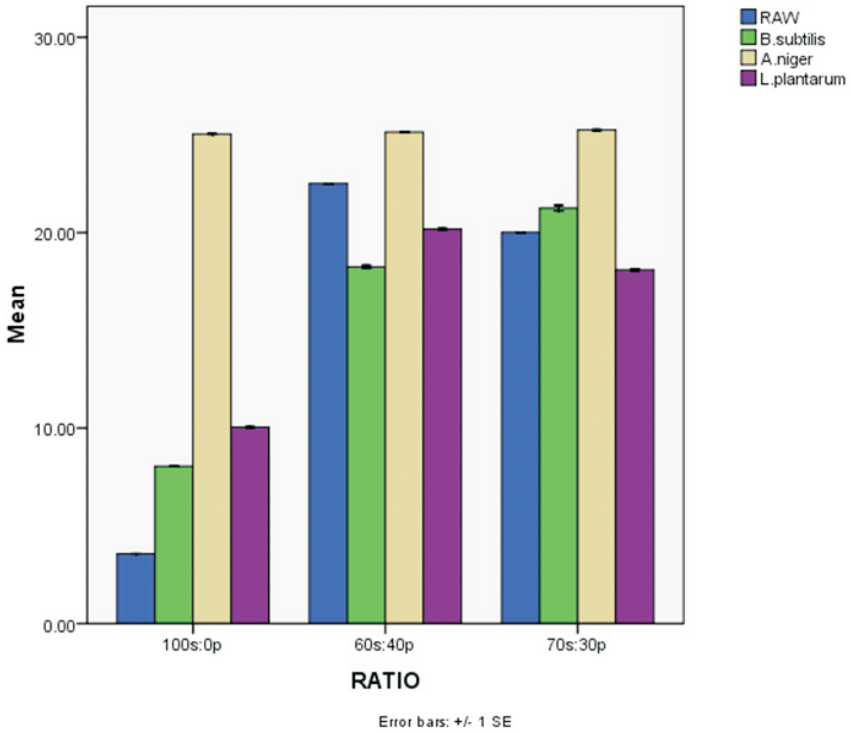

Figure 3: Fat content (%) of sorghum unfortified and fortified with pumpkin before and after fermentation (72hr)
Bars are presented as Mean ±S.E of replicates (n=3)
Key: s= sorghum, p= pumpkin

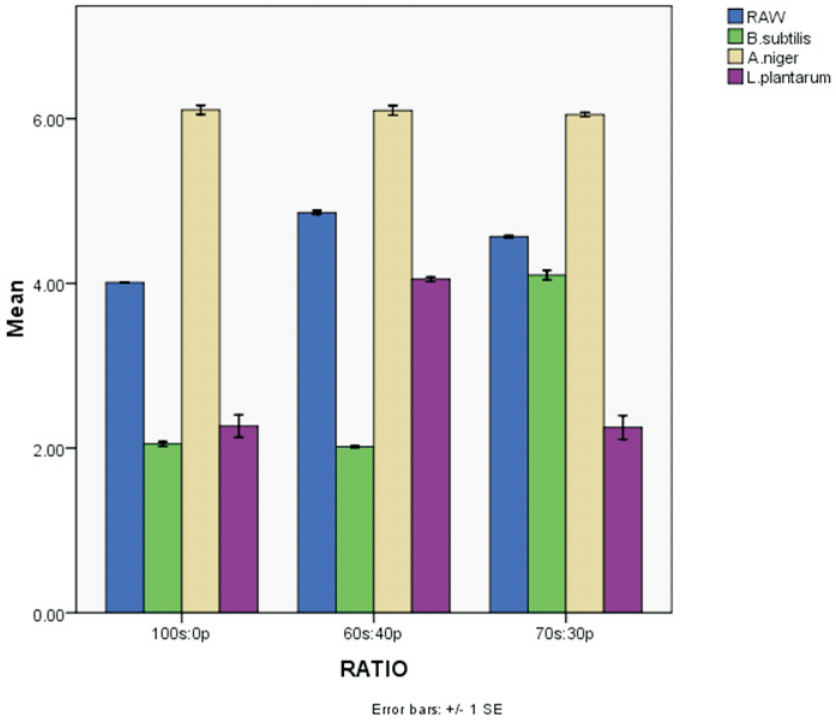

Figure 4: Ash content %) of sorghum unfortified and fortified with pumpkin before and after fermentation (72hr)
Bars are presented as Mean ±S.E of replicates (n=3)
Key: s= sorghum, p= pumpkin

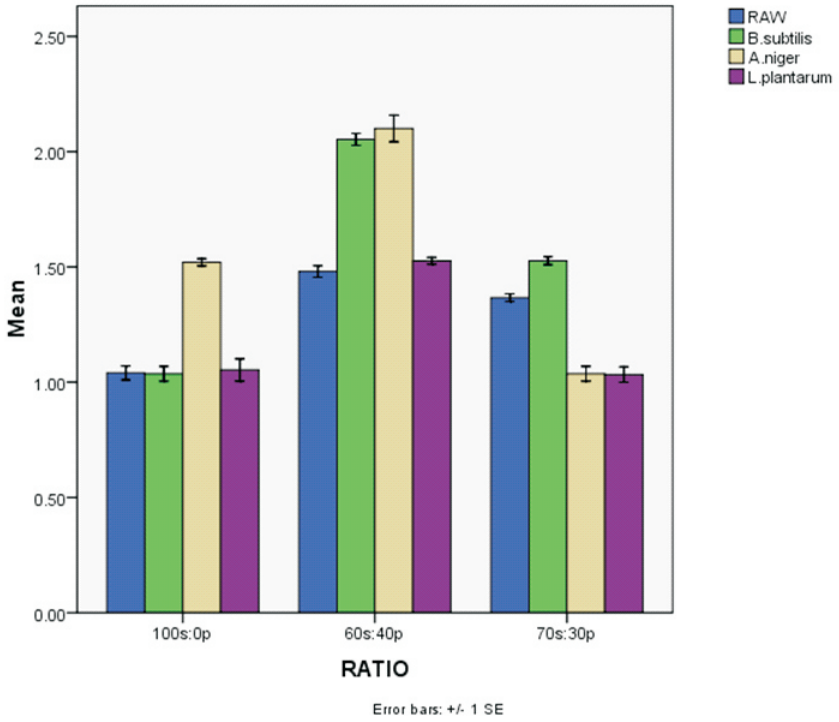

Figure 5: Fibre content (%) of sorghum unfortified and fortified with pumpkin before and after fermentation (72hr)
Bars are presented as Mean ±S.E of replicates (n=3)
Key: s= sorghum, p= pumpkin

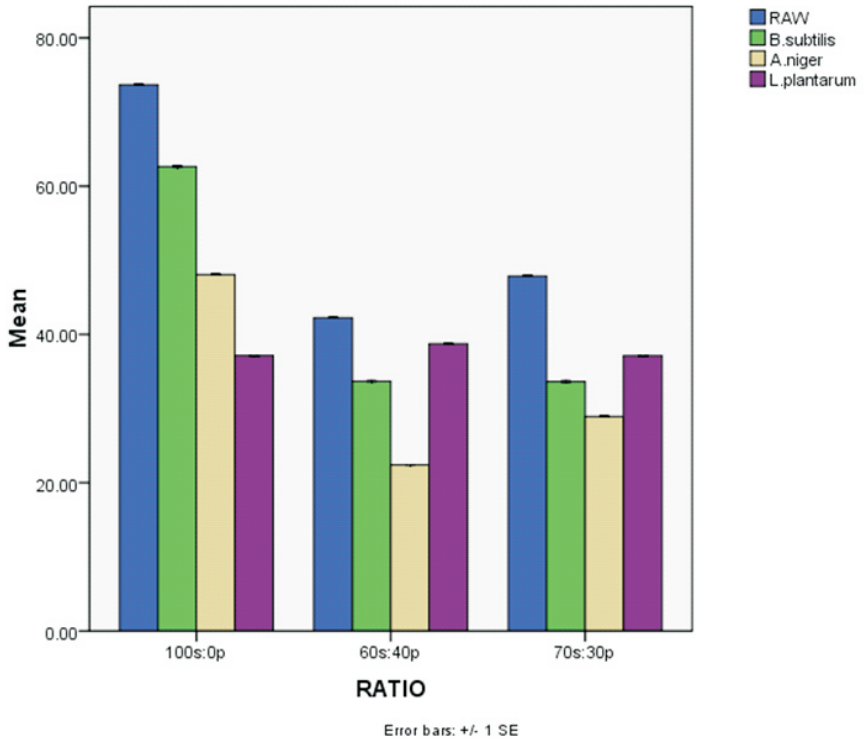

Figure 6: soluble carbohydrate (%) of sorghum unfortified and fortified with pumpkin before and after fermentation (72hr)
Bars are presented as Mean ±S.E of replicates (n=3)
Key: s= sorghum, p= pumpkin

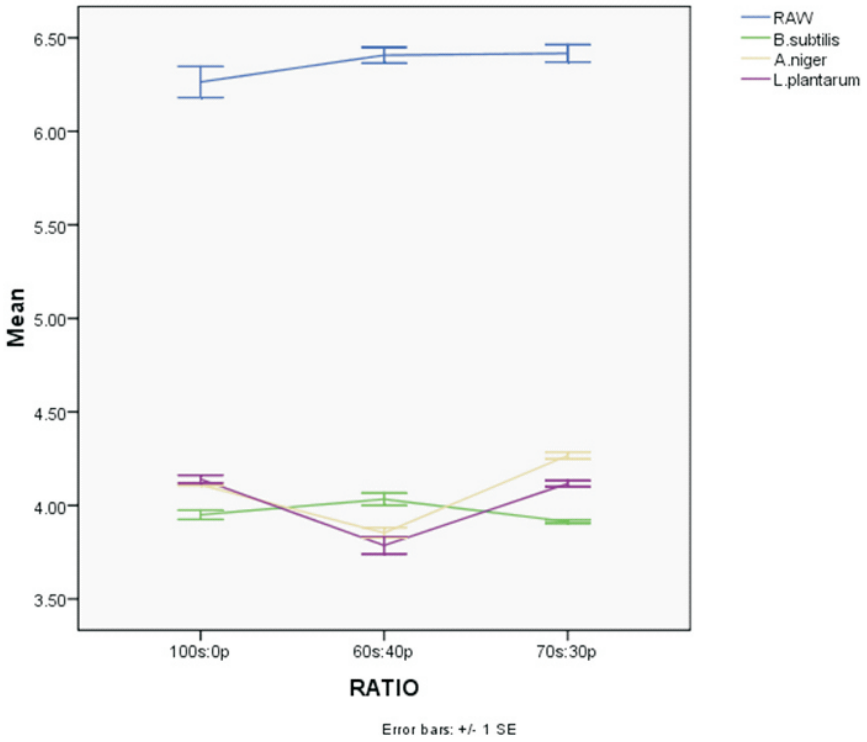

Figure 7: Changes in pH of sorghum unfortified and fortified with pumpkin before and after fermentation (72hr)
Bars are presented as Mean ±S.E of replicates (n=3)
Key: s= sorghum, p= pumpkin

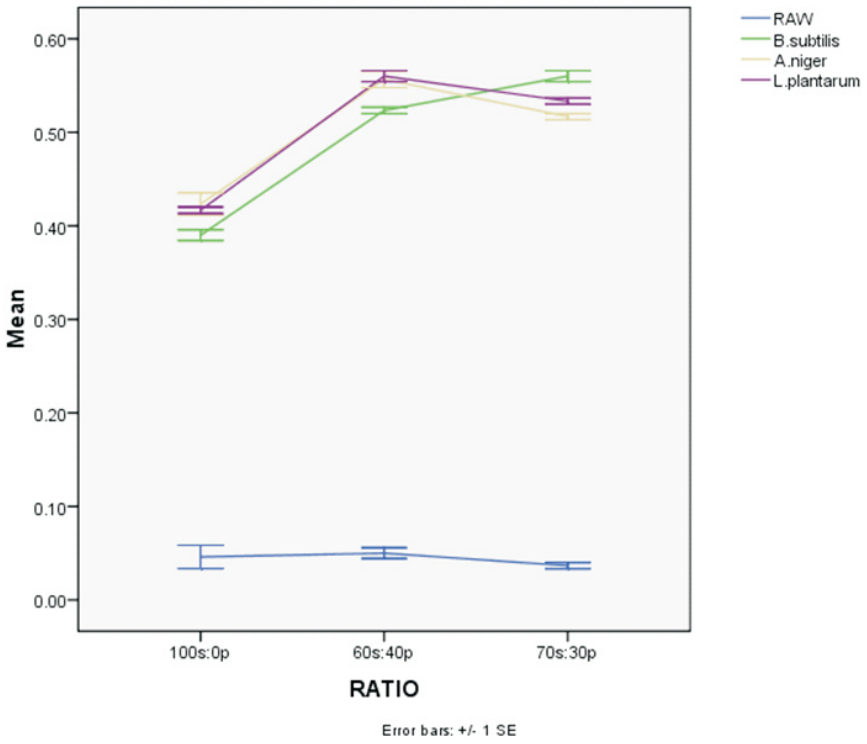

Figure 8:Changes in Total titratable acidity (mg lactic acid/g) of sorghum unfortified and fortified with pumpkin before and after fermentation (72hr)
Bars are presented as Mean ±S.E of replicates (n=3)
Key: s= sorghum, p= pumpkin

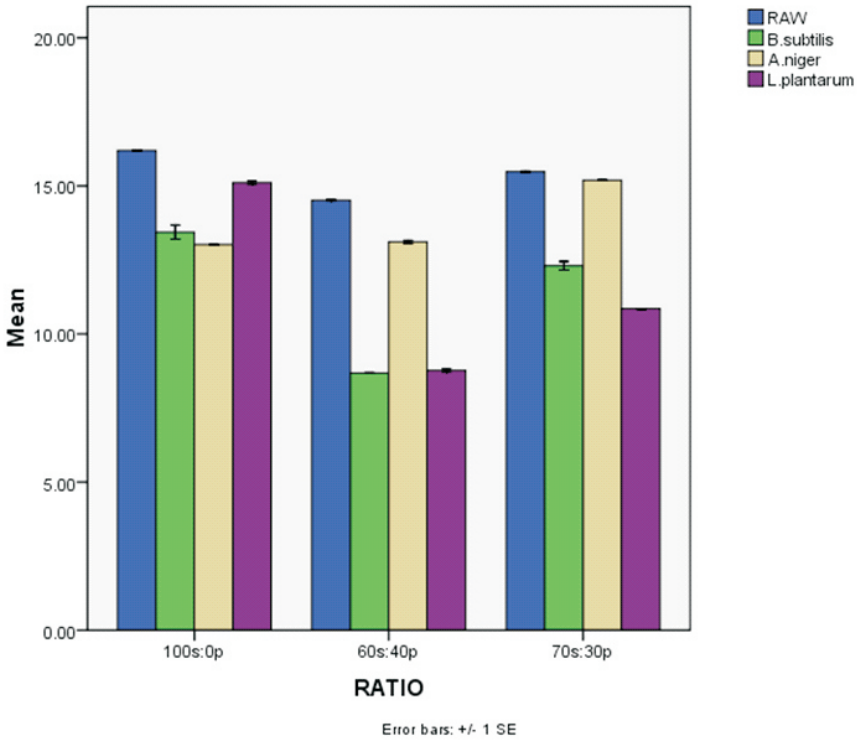

Figure 9: Hydrogen cyanide (mg/g) content of sorghum unfortified and fortified with pumpkin before and after fermentation (72hr)
Bars are presented as Mean ±S.E of replicates (n=3)
Key: s= sorghum, p= pumpkin

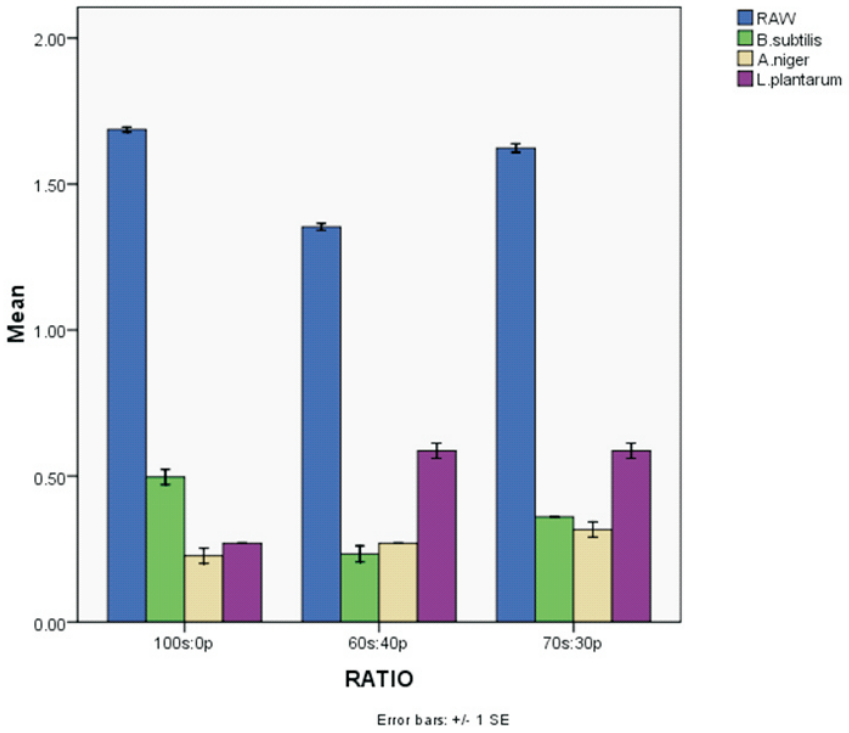

Figure 10: Oxalate content (mg/g) of sorghum unfortified and fortified with pumpkin before and after fermentation (72hr)
Bars are presented as Mean ±S.E of replicates (n=3)
Key: s= sorghum, p= pumpkin

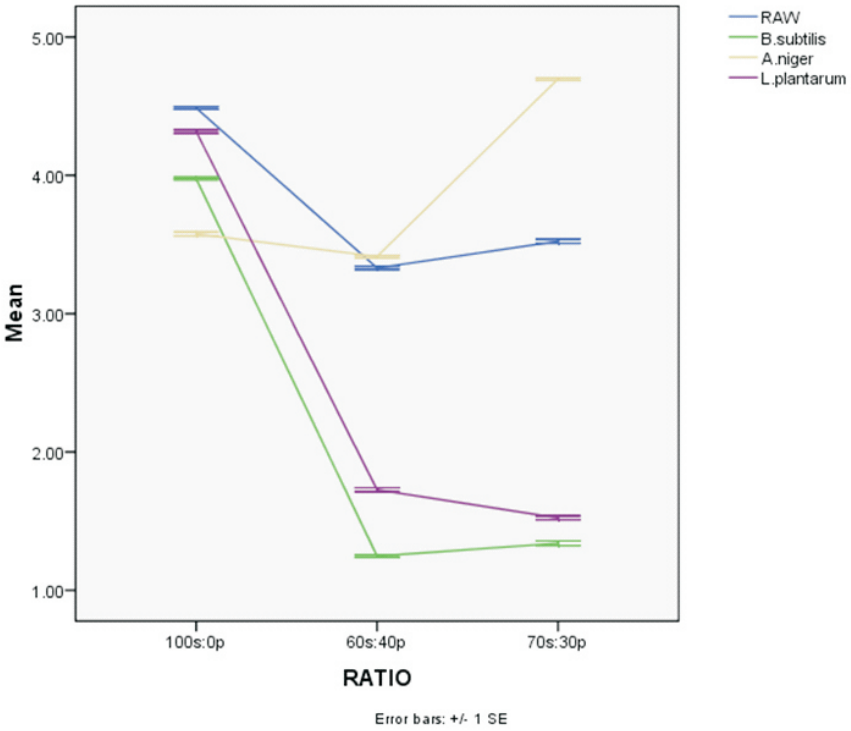

Figure 11: Phenol content (mg/g) of sorghum unfortified and fortified with pumpkin before and after fermentation (72hr)
Bars are presented as Mean ±S.E of replicates (n=3)
Key: s= sorghum, p= pumpkin

DISCUSSION

No change was observed in the moisture content of the sorghum fortified with pumpkin before fermentation but there were changes in the moisture content after fermentation. Unfortified sorghum flour fermented with *Lactobacillus plantarum* had the highest moisture content followed by *Bacillus subtilis* and *Aspergillus niger*. Sorghum fortified in ratio 60s:40p had a higher moisture content, compared to that of 70s:30p. The increase in the moisture content could be attributed to the addition of water to the composite flours prior to fermentation. This is in line with the findings of Oyetoro *et al.* (2013) who reported an increase when sorghum was fortified with walnut and ginger. It should be pointed out that when flours are allowed to equilibrate for a long period of time at 60% relative humidity and at room temperature (25 to 27°C), moisture content might increase.

Crude protein increased after fermentation. Highest protein content was observed at 60s:40p fermentation with *A. niger* and *B. subtilis* followed by 70s:30p after fermentation with *L. plantarum* and *B. subtilis*. A high protein was observed when the sorghum was fortified with pumpkin and also fermented with proteolytic microorganisms. The high protein observed during fortification of the sorghum with pumpkin may be because pumpkin is a protein-rich legume (Usha *et al.,* 2010). This agrees with the work of Ashaye *et al.* (2001) who reported an increase in protein content when yam flour was substituted with 40% cowpea flour.

Fat content increased after fermentation. The highest increase was observed when fermented with *A.niger*. High fat content was observed when the sorghum was fortified with pumpkin, which later increased after fermentation. This is in agreement with the findings of Oyetoro *et al.*, (2013) who reported an increase when sorghum was fortified with walnut and ginger. The variation observed in the ash content during fermentation may be as a result of the activities of the fermenting organisms.

Crude fibre content increased when fortified with pumpkin. This is as a result of the high fibre content of pumpkin (Adebayo *et al.*, 2013). Variation observed in the content after fermentation may be due to the activities of the

fermenting organisms.

Soluble carbohydrate content of the sorghum decreased after fermentation. Carbohydrate content varied and decreased with addition of pumpkin before and after fermentation. This is in agreement with the findings of Oyetoro *et al.* (2013) who reported a decrease in carbohydrate content during sorghum fortification with walnut and ginger. The reduction in carbohydrate levels agrees with the work of Oyewole and Odunfa (1989) that carbohydrate level during fermentation decreases because of the activities of the fermenting microbes.

pH decreased while the TTA increased after fermentation due to the activities of the fermenting organisms. The changes in pH and TTA could be attributed to the production of organic acids from available nutrients by fermenting microorganisms (Ojokoh, 2005). Sample fermented with *L. plantarum* and *A. niger* had more reduced pH compared to that of *B. subtilis.*

Hydrogen cyanide content varies due to the activities of the fermenting organisms, while oxalate content decreased after fermentation. This agreed with the report of Chima *et al.* (2012) who reported a decrease during malting of sorghum grist. Phenol content reduced significantly in sorghum fortified with pumpkin after fermentation with *A. niger.* The anti-nutrients were greatly reduced in the samples fermented with *A. niger* This may be due to the processing that the samples were subjected to coupled with activities of the microbial enzymes involved in the fermentation. The study therefore reveals that fermentation can enhance the nutritional quality of sorghum and pumpkin blends positively

CONCLUSION

The results from this study show that fermentation of pumpkin-sorghum flour blends using pure strains of *Lactobacillus plantarum, Aspergillus niger* and *Bacillus subtilis* improves the nutritional qualities of the blends. Pumpkin fortification affected the fermentation of sorghum. This implies that fermented pumpkin-sorghum flour blends have potentials in the formulation of weaning foods for the management of Protein-Energy Malnutrition (PEM).

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