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INFLUENCE OF STARTER COCKTAIL ON THE NUTRITIONAL QUALITY OF STARTER-DEVELOPED FERMENTED SORGHUM-COWPEA WEANING BLENDS

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ABSTRACT

Lactic acid bacteria and Yeast are known to play a major role in cereal fermentation. The present study aimed at improving the nutritional quality of infant weaning food through cocktail starter fermentation. Sorghum-cowpea blends was fermented with four cocktail starter cultures of lactic acid bacteria (LAB) and Yeast: *Lactobacillus plantarum* MK-1 and *Saccharomyces cerevisiae* MK-Y (AB1), *Lactobacillus plantarum* MK-1 and *Pediococcus acidilactici* MK-3 (AB2), *Pediococcus acidilactici* MK-3 and *Saccharomyces cerevisiae* MK-Y (AB3), and *Lactobacillus plantarum* MK-1, *Pediococcus acidilactici* MK-3 and *Saccharomyces cerevisiae* MK-Y (AB4) for 72 h and was analyzed 12 hourly for microbial load, mineral and vitamin contents. The total viable count of fermenting LABs increased to 5.8 x 10^9 Cfu/g in sample AB1 while the yeast increased to 9.0 x 10^9 Cfu/g in sample AB4. Mineral and vitamin contents showed an increased production with increase in fermentation except for Zinc. The Calcium content was maximally improved to 91 mg/100g in sample AB4. Sample AB1 produced the maximum riboflavin (1.0 mg/100g) and thiamine (0.9 mg/100g) content after 72 h fermentation. The vitamin values were above the recommended daily intake of 0.4 and 0.2 mg/day prescribed in the Reference Nutrient Intakes (RNIs) for infants.Weaning food produced from *L. plantarum* MK-1 and *S. cerevisiae* MK-Y (AB1) led to approximately 2 to 3-fold increase of riboflavin and thiamine content. Therefore, the potential for utilization of the cocktail starter (AB1) fermented sorghum-cowpea, blends as functional weaning food for health promotion exists.

Keywords: Cocktail starter culture, nutritional quality, sorghum-cowpea, weaning food

RÉSUME

Les bactéries lactiques et les levures sont connues pour jouer un rôle majeur dans la fermentation des céréales. La présente étude visait à améliorer la qualité nutritionnelle des aliments de sevrage pour nourrissons grâce à la fermentation en démarreur de cocktails. Les mélanges de sorgho-niébé ont été fermentés avec quatre cultures de démarrage de cocktails de bactéries lactiques (LAB) et de levure: *Lactobacillus plantarum* MK-1 et *Saccharomyces cerevisiae* MK-Y (AB1), *Lactobacillus plantarum* MK-1 et *Pediococcus acidilactici* MK-3 (AB2) , *Pediococcus acidilactici* MK-3 et Saccharomyces cerevisiae MK-Y (AB3), et *Lactobacillus plantarum* MK-1, *Pediococcus acidilactici* MK-3 et *Saccharomyces cerevisiae* MK-Y (AB4) pendant 72 h et a été analysé toutes les 12 heures pour la charge microbienne, minéral et les teneurs en vitamines. Le nombre total viable de LAB en fermentation a augmenté à 5,8 x 109 Cfu / g dans l'échantillon AB1 tandis que la levure a augmenté à 9,0 x 109 Cfu / g dans l'échantillon AB4. Les teneurs en minéraux et en vitamines ont montré une production accrue avec une augmentation de la fermentation, sauf pour le Zinc. La teneur en Calcium a été améliorée au maximum à 91 mg / 100 g dans l'échantillon AB4. L'échantillon AB1 a produit la teneur maximale en riboflavine (1,0 mg / 100 g) et en thiamine (0,9 mg / 100 g) après 72 h de fermentation. Les valeurs de vitamines étaient supérieures à l'apport quotidien recommandé de 0,4 et 0,2 mg / jour prescrit dans les Apports nutritionnels de référence (RNI) pour les nourrissons.Les aliments de sevrage produits à partir de *L. plantarum* MK-1 et *S. cerevisiae* MK-Y (AB1) ont conduit à augmentation d'environ 2 à 3 fois de la teneur en riboflavine et en thiamine. Par conséquent, il existe un potentiel d'utilisation des mélanges de sorgho-niébé fermentés (AB1) comme aliment de sevrage fonctionnel pour la promotion de la santé.

INTRODUCTION

Cereals and legumes, utilized individually or as composites, are the main sources of nutrients for weaning children in developing countries. Due to their abundance and acceptability, cowpeas *(Vigna unguiculata)* varieties are options for use in weaning foods in Africa. Sorghum (*Sorghum bicolor* L. Moench) as "Ogi" is a staple food in most parts of Nigeria, where it is the main source of carbohydrates and proteins for millions of people. Like other cereal products, sorghum products have poor nutritional value. This is due to their nutritional deficiencies in lysine and tryptophan, unlike animal proteins (Omole *et al*., 2017) and the presence of anti-nutritional factors, such as tannins and phytates, which interact to form bonds with proteins, vitamins and minerals, thus, restricting their bio-availability (Nkhata *et al.*, 2018). The above factors contribute to nutritional deficiency, which may result in different kind of diseases, as a result of its consumption level.

Formulation of healthy weaning food that is rich in amino acids, minerals and vitamins could potentially protect infants from health risk. According to previous studies, the unhealthy state of adulthood is determined by environmental factors and insufficient nutritional value of food taken during childhood (Gubbels, 2020). Thus, Macro- and micro- nutrients are essential for the growth and development of weaning children. It was previously reported that, zinc deficiency lead to functional consequence such as weight loss, compromised physical growth, reproductive problem and low immunity (Gupta et al., 2020). Also, deficiency in riboflavin results into riboflavinosis. Various techniques have been utilized to improve the protein digestibility and mineral availability of sorghum amongst which is fermentation. Fermentation is widely used in a traditional way, for processing sorghum, and fermented products which are well accepted and widely used, as complementary foods. Fermentation could result in breakdown of the mineral-antinutrient complexes, through hydrolysis by digestive enzymes. The fermentation of millet and sorghum has been reported to increase the Iron, Calcium and Potassium availability (Udeh *et al.*, 2018). In addition, germination was reported to improve the availability of Iron, Zinc and Calcium of Sorghum- based complementary food (Nkhata *et al.*, 2018).

Fermentation improves the protein and mineral quality of non-alcoholic sorghum beverage (Adeyanju *et al.*, 2019). Nkhata *et al.* (2018), reportedly explained the mechanism by which fermentation increases mineral bioavailability either through reduction in phytic acid that binds minerals making them free and more available. Loss of complex matrix, that embeds minerals or as in iron absorption at low pH during fermentation by conversion of ferrous iron to ferric iron, which is readily absorbed. Moreover, fermentation also provides optimum pH for enzymatic degradation of phytate. Their low product pH also confers the advantage of microbiological safety. In the natural fermentation of sorghum flour for "Ogi", several aerobic, mesophilic, and lactic acid bacteria were involved and *Lactobacillus plantarum*, *Pediococcus acidilactici* and *Saccharomyces cerevisiae* were reported to play a major role in sorghum flour fermentation (Kawthar *et al*., 2018). Hence, the use of the cocktail of these bacteria as starters for fermentation becomes imperative. The use of starter cultures would be important to obtain a predictable end product with a desired quality.

The improvement in protein quality of cereals has been carried out, though combination with protein rich sources. Soy bean protein has been included in sorghum flour as protein and mineral supplementation (Asuk *et al*., 2020). In the previous studies, supplementation of sorghum flour with cowpea to improve the protein quality of weaning food was investigated (Wakil and Kazeem, 2012). However, there is no report on the effect of the starter cocktail the on the mineral and vitamin contents of the formulated sorghum-cowpea weaning blends. The present study therefore, assessed the effect that the different starters on the improvement of nutritional quality, through monitoring of the mineral and vitamin contents of the cocktail starter- fermented weaning blends.

MATERIALS AND METHODS

Sample collection

Brown Sorghum *(Sorghum bicolor)* and brown cowpea *(Vigna unguiculata)* (Ife brown) were purchased from Institute of Agricultural Research and Training (IAR&T), Moor Plantation, Ibadan, South western Nigeria. They were transported to the laboratory in cleaned polyethylene bags for further use.

Organisms and culture methods

Pure cultures of *Lactobacillus plantarum* MK-1 and *Pediococcus acidilactici* MK-3 were obtained from the Industrial and Biotechnology Laboratory of the Department of Microbiology, University of Ibadan, Nigeria. The young cultures of *L. plantarum* MK-1 *and P. acidilactici* MK-3 were routinely maintained on de man Rogosa and Sharpe (MRS) broth pH 5.5 at 30°C. The yeast (*Saccharomyces cerevisiae* MK-Y) was isolated from "Ogi" and routinely maintained on Malt Extract Agar (MEA) at 30°C

Processing of cereal and legume samples

The cereal grains *(Sorghum bicolor*) was freed from dirt and extraneous materials, by manual sorting and washed thoroughly with sterile distilled water. It was then oven- dried at 50°C for 8 h, dry milled and sieved to obtain a fine particle size 0.5 mm. They were packed in clean polyethylene bags and stored at 4°C pending further use. The legume sample, Ife brown *(Vigna unguiculata)* was freed from dirt and extraneous materials by manual separation, washed thoroughly in sterile distilled water, followed by dehulling and oven drying at 50°C for 48 h, dry milling and sieving to obtain fine flour. They were packed into clean polyethylene bags and stored at 4°C until use.

Formulation of composite blends and fermentation

The sorghum and cowpea flour blends were mixed in ratio 70:30 w/w (Wakil and Ola, 2018). Each formulated composite blend in the fermenting flask was made into slurry (30%; w/v). One liter of each slurry was inoculated aseptically with 1 ml of the inoculum starter organisms containing 2.0 x 10^9 cfu/ml of *L. plantarum* MK-1 and *P. acidilactici* MK-3 and 2.8 x 10^8 cfu/ml of *Saccharomyces cerevisiae* MK-Y with different combinations as shown in Table 1. The inoculation was accompanied by stirring using a sterile glass rod. Fermentation was allowed to hold for 72 h and sampling was carried out every 12 h.

Determination of mineral and vitamin contents of fermenting blends

The mineral (Calcium, Iron and Zinc) content of the fermenting blends was determined by AOAC (1980). Briefly, 2 g of samples were ashed at 550°C furnace for 2 h. To the ash, 10 ml of 0.1 N HCL was added. The resultant solution was warmed on a heater for few seconds to avoid floating and filtered through, Whatman No. 2 filter paper into another 100 ml flask. Distilled water was added to the solution to make up 100 ml. The resulting solution was used for analysis of mineral element by using an atomic absorption spectrophotometer (Analytical Jend, Contra 700, Germany).The vitamin (Riboflavin, Thiamine and Niacin) were determined by spectrophotometric method (AACC, 1980). Riboflavin content was determined by extracting 5 grams of ground sample in sulfuric acid. The impurities were oxidized with permanganate and the intensity of the extract fluorescence was determined by a fluorimetric measurement. In the case of thiamine, the sample was oxidized to thiochrome by using alkaline potassium ferricyanide, after treatment of sample with starch-and protein-degrading enzymes was carried out. The resulting fluorescent thiochrome was extracted in isobutanol and measured by a fluorimetric measurement. Niacin was determined chemically by using a colorimetric technique. Five grams of ground sample was extracted in alkaline medium, clarified, and reacted with sulfanilic acid and cyanogen bromide to yield a yellow color.

RESULTS

Total LAB and yeast viable count of starter-fermented Sorghum-cowpea blends

Table 1 shows the total viable count of Lactic acid bacteria (LAB) and yeast relative to fermentation time. The LAB counts showed increment by all the starter cocktail tested throughout the fermentation time. The highest LAB count (5.8 x 10^9 cfu/g) was observed by 72 h in the sample fermented with cocktail of *Lactobacillus plantarum* MK-1 and *Saccharomyces cerevisiae* MK-Y (AB1), followed by sample AB4 (*L. plantarum* MK-1, *P. acidilactici* MK-3 and *Saccharomyces cerevisiae* MK-Y), which had the LAB count of 3.2 x 10^9 cfu/g, while the lowest LAB count of 2.5 x 10^9 cfu/g was obtained in the sample fermented with cocktail cultures of *P. acidilactici* MK-3 and *S. cerevisiae* MK-Y (AB3) after 72 h. Likewise, the yeast count increased with increase in fermentation time and later decreased by 48 h with the highest yeast count (9.0 x 10^9 cfu/g) recorded in sample AB4 at 36 h, followed by sample AB1. Sample AB3 had the least yeast count at 8.2 x 10^8 cfu/g at the same fermentation time. However, sample AB1 and AB3 showed similar responses in terms of the LAB and the yeast count.

Table 1. Total lactic acid bacteria and yeast viable count of cocktail starter fermented sorghum-cowpea blends.

Sample code	Total viable count (Cfu/g)						
	Fermentation time (h)						
	0	12	24	36	48	60	72
	Lactic acid bacteria						

AB1	3.2×10^8	9.7×10^8	1.0×10^9	1.0×10^9	1.8×10^9	2.7×10^9	5.8×10^9
AB2	3.0×10^8	6.5×10^8	7.6×10^8	8.0×10^8	9.0×10^8	1.1×10^9	2.8×10^9
AB3	2.6×10^8	3.6×10^8	6.0×10^8	7.2×10^8	1.2×10^9	1.4×10^9	2.5×10^9
AB4	2.5×10^8	4.0×10^8	7.5×10^8	1.0×10^9	1.8×10^9	2.5×10^9	3.2×10^9
				Yeast			
AB1	3.0×10^8	5.2×10^8	1.0×10^9	1.2×10^9	1.4×10^9	9.0×10^8	2.3×10^8
AB3	2.5×10^8	3.6×10^8	6.0×10^8	8.2×10^8	1.2×10^8	6.0×10^8	1.5×10^8
AB4	2.6×10^8	4.0×10^8	7.0×10^8	9.0×10^9	1.5×10^9	9.7×10^8	3.0×10^8

Sample interpretation

AB1 - *L. plantarum* MK-1 *and S. cerevisiae* MK-Y

AB2 - *L. plantarum* MK-1 *and P. acidilactici* MK-3

AB3 - *P. acidilactici* MK-3 *and S. cerevisiae* MK-Y

AB4 - *P. acidilactici* MK-3, *L. plantarum* MK-1 *and S. cerevisiae* MK-Y

Mineral composition of cocktail starter- fermented sorghum-cowpea blends

The analysis of mineral content of the weaning blends fermented with different starter cocktail shows that, the Calcium content increased with increasing fermentation time while the Zinc content decreased with increase in the fermentation. The Calcium content of the starter fermented weaning blends is shown in Figure 1. At 72 h, the Calcium content reached its maximum in sample AB4, followed by sample AB3, while the least was observed in sample AB1. From the figure, the sample fermented with mixed cultures of the three organisms (AB4), had calcium content of 91.0 mg/100 g after 72 h, while the lowest Calcium content (79.0 mg/100 g) was observed in the sample AB1 by the same fermentation time. Although, a comparable Calcium content was observed in sample AB1, AB2 and AB3 from 60 h to 72 h .

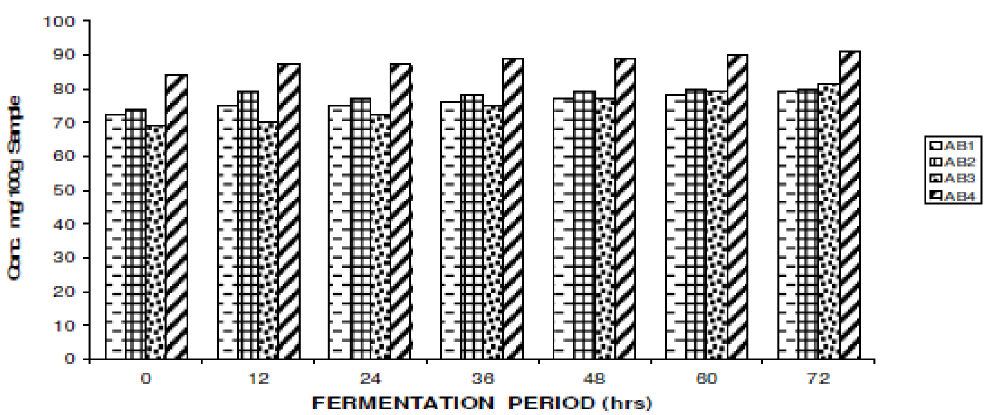

Figure 1. Effect of fermentation periods on Calcium content of cocktail starter -produced sorghum-cowpea blends.

Key: as in Table 1

Figure 2 shows that the zinc content for all the combinations of the fermented formulated blends was stable for the first 12 h, and later decreased gradually till the end of the fermentation. Sample AB2 had the highest Zinc content (0.094 mg/100 g), followed by sample AB3, while sample AB1 and AB4 had the least Zinc content (0.090 mg/100 g) after 72 h of fermentation. Similarly, the highest Iron content was recorded in sample (AB2) at every fermentation time (results not shown).

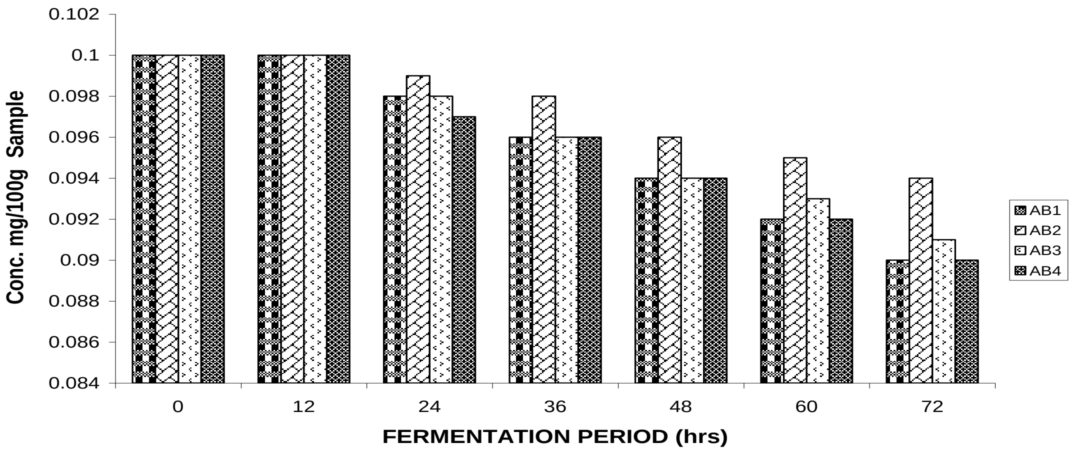

Figure 2. Effect of fermentation periods on zinc content of cocktail starter-produced sorghum-cowpea blends.
Key: As in Table 1

Vitamin composition of starter cocktail fermented sorghum-cowpea blends

The vitamin contents of all the cocktail starter-fermented blends showed increase in riboflavin and thiamine with increased fermentation time (Figure 3). From the figure, sample AB1 had the highest riboflavin content throughout the fermentation time, while the least was observed in the sample AB3. The riboflavin content increased to 1.0 mg/100g in sample AB1 while the samples AB2 and AB3 progressively increased to 0.7 mg/100g and 0.6 mg/100g, respectively. Generally, sample AB1 showed great improvement in the riboflavin content throughout the fermentation periods followed by sample AB4.

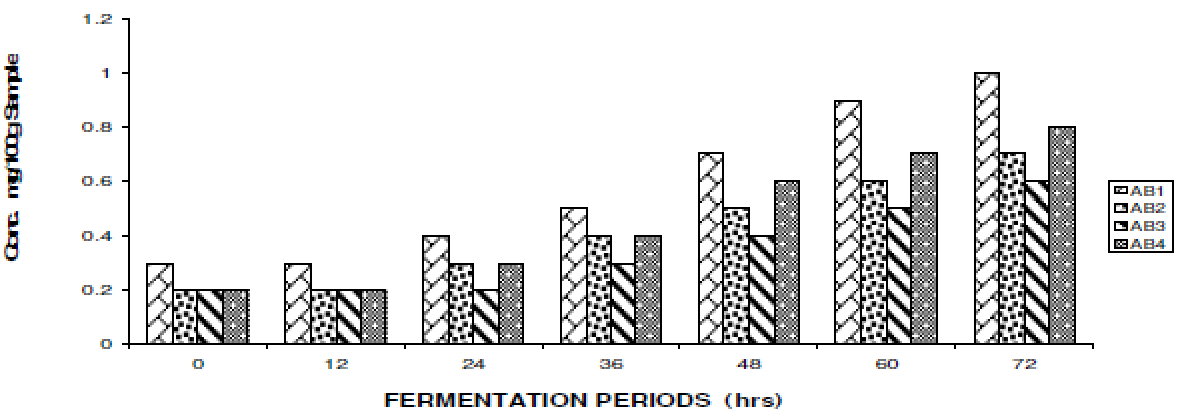

Figure 3. Effect of fermentation periods on riboflavin content of cocktail starter -produced sorghum-cowpea blends
Key: As in Table 1

The effect of starter cocktail on thiamine content of fermented sorghum-cowpea blends (Figure 4) shows that, the thiamine content of all the starter samples reached maximum at 48 h. The highest thiamine content (0.9 mg/100 g) was recorded in sample AB1 followed by sample AB4, while the sample AB3 at 48 h recorded the lowest thiamine content of 0.6 mg/100 g. As for sample AB2, the thiamin content was only improved to 0.7 mg/100 g. Similar to the riboflavin content, the thiamine content was greatly enhanced by sample AB1.

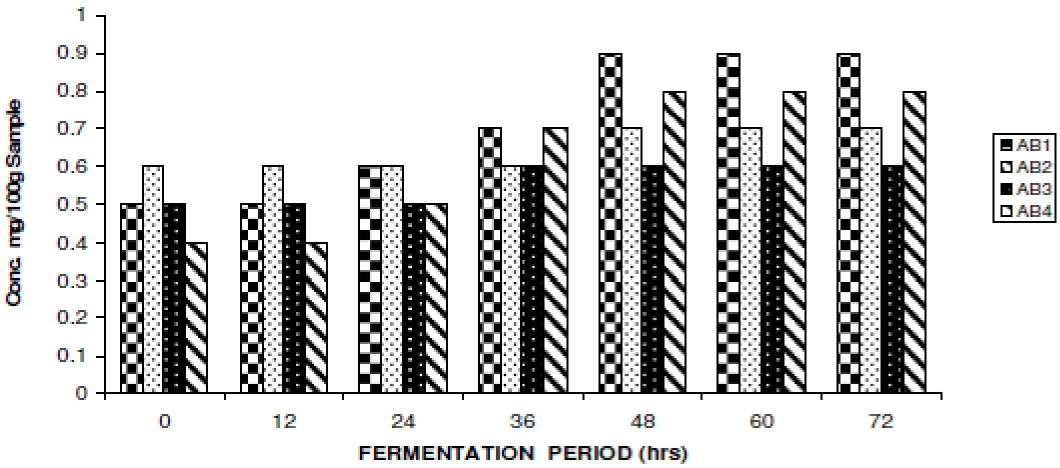

Figure 4. Effect of fermentation periods on thiamine content of cocktail starter-produced sorghum–cowpea blends
Key: As in Table 1

DISCUSSION

Species of Lactic acid bacteria and yeast were largely involved in the fermentation of cereals and majority of them were isolated and used as starter culture in fermentation. However, exploring starter cocktail for species of lactic acid bacteria and yeast previously involved in uncontrolled fermentation of “Ogi” (A local Nigerian weaning food), might serve as a promising method for producing nutritionally beneficial and functional weaning food. In the current study, some starter candidates were selected and the contribution of their cocktails to the nutritional value of sorghum-cowpea fermented weaning blends were observed. From the study, the lactic acid bacteria and yeast count increased with fermentation time. Concomitant increase in lactic acid bacteria and yeast has been observed before, and the association of lactic acid bacteria and yeast has been noted in several fermented cereal foods (Voidarou *et al.*, 2021; Houngbédji *et al.*, 2018; Wakil and Onilude, 2009). In fact, the development of lactic acid bacteria is stimulated by yeast, which provides soluble nitrogen compounds and other growth factors (Hassen *et al.*, 2018). The interaction between *Lactobacillus hordei* and *yeasts* is created by the release of vitamin B complex and amino acids released from *Saccharomyces cerevisiae* and *Zygotoluraspora florentina*, likewise, the growth of *Lactobacillus negalii* is supported by the secretion of arginine by the yeasts (Stadie *et al.*, 2013). However, compared to the LAB count, the yeast count observed a decrease at 60-72 h of fermentation period. The lower yeast count could suggest mechanism such as: autolysis or yeast cell reaction to some metabolites probably produced by the LABs. In addition, the excessive accumulation of soluble sugars i.e. glucose could also result in feedback inhibition of the yeast population. Similar decrease in *G. geotrichum* cell count was observed during co-culture with *L. plantarum* and *E. faecalis* in milk fermentation (Chaves-López *et al.*, 2017). In fact, Stadie *et al.* (2013) suggested that, yeast autolysis or triggering selective release of nutrients are major mechanisms involve in the co-culture of LAB and yeast.

The interaction of minerals and antinutritional factors are major factors obstructing the bioavailability of minerals. According to this study, the mineral content of the formulated blends revealed that, Calcium content was enhanced as the fermentation period was progressing, while the Zinc content was declined. This result is similar to that of Adegbehingbe (2015), who observed that, fermentation of sorghum-Irish potato with starter cultures resulted to increased mineral availability, when compared to uncontrolled fermentation. The reduction in Zinc content observed in this study is similar to the report of Afify *et al.* (2011) who reported that, up to 30% of Zinc content of sorghum was lost to soaking, which was attributed to the leaching of Zinc into the soaking medium. In contrast to this observation, fermentation was more effective in increasing the bio-accessibility of iron and zinc (Muleya Née Gabaza *et al.*, 2017). Improvement in calcium content of this study is similar to that of Onwurafor *et al.* (2014), who reported increase in Calcium and Iron content in the fermentation of mung bean flour (Vigna radiata), with back slopping than in spontaneous fermentation. The fermentation of sorghum grains was used to increase the Sodium, Calcium, Iron and Phosphorus and Magnesium content (Adebo, 2020). Wakil and Ola (2018), reported increase in calcium and iron content in the fermentation of maize-tiger nut weaning food with *consortium of Lactobacillus plantarum*, than in spontaneous fermentation period. Bioavailability of Calcium could be as a result of the enzymatic activities, produced by the fermenting starters, which hydrolyses the substrates and breakup the complex that was formed between the minerals and the antinutrients, thereby increasing the bioavailability of Calcium ion. The reduction in Zinc content during the period of fermentation, may be as a result of the activities of the microorganism, which might have utilized the Zinc ion for their metabolic activities after been cleaved from the antinutrient–mineral complexes. Therefore, Zinc ion might be an essential mineral required by the cocktail starters for metabolism. The increase in Calcium content of the cocktail starter fermentation is of significant importance to weaning children. Calcium is required to build and develop healthy bone, teeth and for prevention of bone diseases such as, rickets and osteorosporosis.

In the analysis of the vitamin content of the fermented formulated blends, the riboflavin and thiamine contents were improving but, depending on the starter cocktail applied. The increase in riboflavin and thiamine in sample AB1 could be nutritionally advantageous to weaning children, as vitamins play significant role in body metabolism. The high riboflavin content could help in the synthesis of coenzyme flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). Improved thiamine content could be nutritionally beneficial in weaning children, since it is implicated in energy production, red blood cell formation and brain functioning (Kerns *et al.*, 2015; LeBlanc *et al.*, 2011). It was also reported by Ratnavathi and Chavan (2016), that, the group B vitamins usually showed increase with fermentation and it could be influenced by the type of raw material used and the fermentation process employed. Several strains of LAB had been shown to synthesize vitamins. For instance, *Lactobacillus fermentum* produced riboflavin for riboflavin enriched bread (Russo *et al.*, 2014). It is worthy to note that, the riboflavin content of the blend fermented with cocktail of *Lactobacillus plantarum* and *Saccharomyces cerevisiae* (AB1) increased by 3- folds. The result agrees with the findings of Capozzi *et al.* (2011), who reported that, riboflavin content increased to about two to three fold in the fermentation bread and paster, respectively. Recently, fermentation of lupin seed for lupin tempeh, observed significant elevated concentrations of Riboflavin synthesized by the co-culture of *Rizopus oryzea* and *Propionibacterium freudenreichi* (Wolkers-Rooijackers *et al.*, 2018). Similarly, a two fold increase in riboflavin was observed, when cocktail of yeast and *Lactobacillus fermentum* PBCC11.5 were applied, as starter culture for production riboflavin fortified functional bread (Russo *et al.*, 2014). Interestingly, the riboflavin content produced from the fermented blends by using the various starter cocktails, falls within the recommended daily intake concentration. The observed increase in Thiamine content is in line with that of Kohajdova, (2017). Soybean fermented with mix culture of *Steptococcus thermophilus* ST5 and *Lactobacillus helveticus* R0052 results in increase in thiamine and pyridoxine concentrations (Champagne *et al.*, 2010). Also, Struyf *et al.* (2017) reported that, fermentation of whole wheat flour with yeast resulted in thiamine, riboflavin enrichment.

CONCLUSION

The use of cowpea as well as cocktail starter cultures, have greatly improved the nutritional contents of the fermented weaning food. The cocktail cultures of *Lactobacillus plantarum* (MK-1) and *Pediococcus acidilactici* (MK-3) (AB2) reasonably improved the mineral and vitamin contents of the fermented formulated weaning blends. Likewise, blend fermented with cocktail starter of *Lactobacillus plantarum* (MK-1) and *Saccharomyces cerevisiae* (MK-Y) (AB1) may be desirable in the production of vitamin rich weaning food. The enhanced mineral and vitamin contents of the cocktail fermented blends achieved in the current study could increase its commercial values and reduce the need for chemical fortification of weaning foods.

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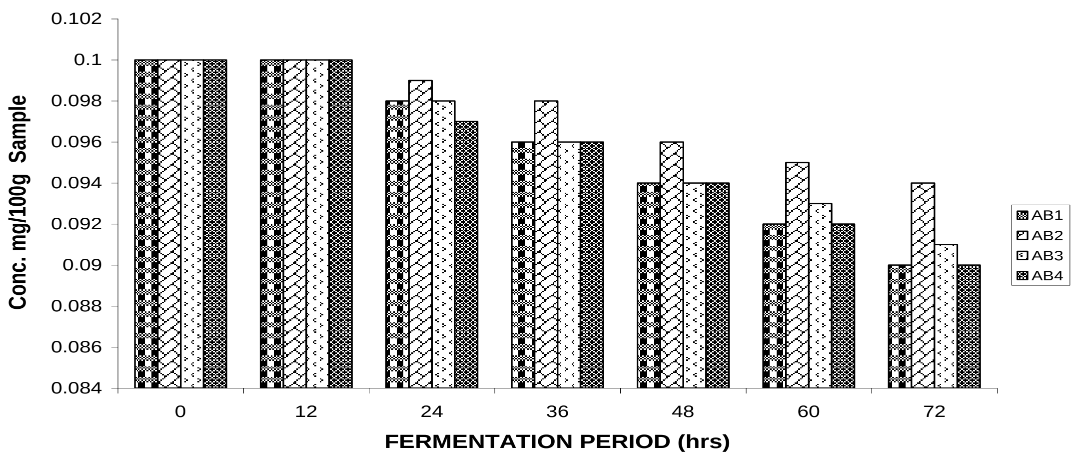

Figure 2. Effect of fermentation periods on zinc content of cocktail starter-produced sorghum-cowpea blends.
Key: As in Table 1

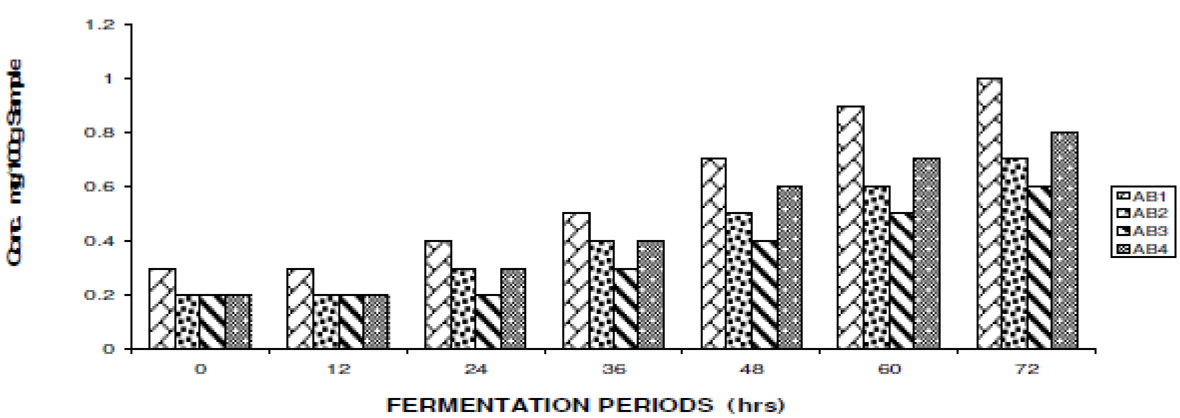

Figure 3. Effect of fermentation periods on riboflavin content of cocktail starter -produced sorghum-cowpea blends
Key: As in Table 1

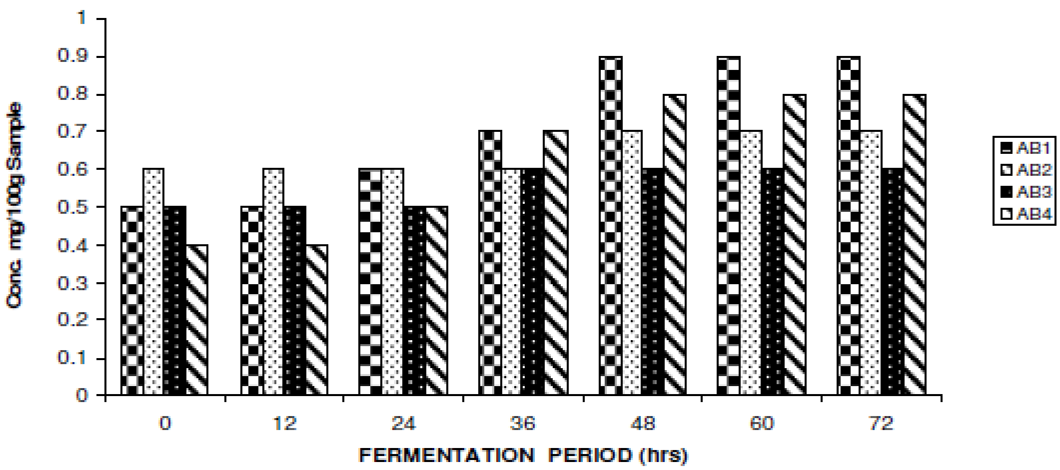

Figure 4. Effect of fermentation periods on thiamine content of cocktail starter-produced sorghum–cowpea blends
Key: As in Table 1