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EFFECT OF FERMENTATION ON THE VITAMIN AND MINERAL CONTENTS OF BAMBARA NUT USING SELECTED RHIZOPUS SPECIES

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ABSTRACT
This study evaluates the effect of fermentation with selected Rhizopus species on the vitamin and mineral contents of bambara nut (Voandzeia subterranean L.thouars) which is a leguminous plant. Bambara nut was fermented with three species of Rhizopus (R. oligosporus, R. nigricans and R. oryzae) and their combinations for 3 days. The samples were collected at 12 hourly intervals and were blanched, dried and milled to produce bambara flour. Vitamin and mineral contents determination were carried out on the samples. The values of vitamin A, riboflavin, niacin and thiamine were (21.70 – 66.14%), (0.38 – 0.86 mg/g), (0.16 – 3.53 mg/g), (0.50 – 4.52 mg/g) and (0.24 – 0.02mg/g), respectively. Mineral contents (potassium, calcium, phosphorus, iron and magnessium) ranges were (1.228 – 1.633), (0.093 – 0.300), (0.297 – 0.465), (0.013 – 0.028) and (0.017 – 0.052mg/100g), respectively. Therefore, fermented bambara nut could find uses in combacting endemic malnutrition in the developing countries.

Keywords**:** Bambara nut, vitamin content, fermentation, mineral content, Rhizopus

INTRODUCTION

Legume seeds are of prime importance in human and animal nutrition particularly in tropical Africa, and especially in Nigeria. This is due mainly to their relatively high protein content (20 – 50%), compared to most cereal grains and root crops (Ustimenko – Bakumovsky, 1993). Bambara nut is leguminous and indigenous crop to Africa. It is of immense nutritional importance in Africa because it contains up to 24% protein and has a good balance of the essential amino acids coupled with relatively high proportions of lysine (6.8%) and methionine (1.3%) although there are some factors that militate its full potential.

Despite the nutritional benefits of bambara nut, it remains underutilised probably because it takes long time to cook, contains antinutritional factors and does not dehull easily. Also, the other major component of the beans is carbohydrate (mainly starch of up to 50%) (Poulter, 1981). Hence, it appears as a promising crop which can improve nutritional and functional properties of food. Consequently, study on selected properties of bambara nut during fermentation with different species of *Rhizopus* primarily has potentials to contribute to making available appropriate food for human consumption towards the eradication of malnutrition in the developing nations of Africa. It also has the potential of reducing child mortality rate which has been estimated to be between 3.7 and 4.3 million annually in Africa (Egounlety and Syarief, 1992; Amadi *et al*., 2004).

Fermenting bambara nut considerably ensures enzymatic splitting of cellulose, hemicellulose and related polymers which are not ordinarily digestible by man into simpler sugars and their derivatives. Other inherent advantages of fermented products include safety, an indication of non-toxicity in terms of being hazardous to man due to long usage, acceptability, reduced cost as influenced by ease of production without expensive equipment and without significant loss of ingredient and long shelf life (FAO, 1988; Alobo and Babalola, 2002). However, there is indication that the benefits of the nut cannot be maximally harnessed without a detailed understanding of the effect of fermentation using different Rhizopus species and their combinations on the vitamin and mineral contents of bambara nut.

MATERIALS AND METHODS

The bambara nut (*Voandzeia subterranean* L. Thouars) used for this study was obtained from a local market in Ogbomoso, Nigeria. The different species of *Rhizopus* for the study were obtained from the Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso, Nigeria and the Institute of Agricultural Research and Training (IAR&T), Ibadan. The chemicals that were used were of analytical grade.

Sample preparation

Subculturing of fungal cultures

The organisms used for fermentation namely; *R. oligosporus*, *R. oryzae* and *R. nigricans* were subcultured by the procedure described by Olanipekun *et al*. (2015): Five hundred millilitres of PDA was prepared by adding 18 g of potato dextrose agar to 500 ml of distilled water, homogenized and sterilized. After cooling, 15 ml of PDA was dispensed into each McCartney bottle and allowed to set in slant form. The pure cultures of *R. oligosporus*, *R. oryzae* and *R. nigricans* were subcultured singly into each McCartney bottle containing PDA and incubated at 30ºC for 4 days.

Preparation of spore inocula

Inoculated slants were incubated for 4 days to allow for sporulation. Spores were then harvested with 100 ml of sterile distilled water and shaken vigorously to dislodge the spores. 1 ml of each spore suspension was taken and transferred into a haemocytometer for spores' enumeration. Two millilitres of these spore suspensions from either *R. oligosporus*, *R. oryzae* or *R. nigricans* were used to inoculate the substrates either singly or in combinations within 0 -72h fermentation period.

Preparation of fermented bambara flour

Bambara nut was fermented according to the method of Olanipekun *et al.* (2015). Bambara nut (4 kg) was cleaned and washed with tap water. It was steeped in water for 24 h and dehulled. The steeped beans were boiled in the steeped water for 15 min, drained and spread out to cool at 30^{o}C for 30 mins as shown in Fig. 1. One hundred grammes of dehulled bambara nut was then poured into the polythene bag that was perforated and a volume of 2 ml of these spore suspensions of either *R. oligosporus* (2.0 x 10^6 cfu/ml) , *R. oryzae* (1.7x 10^6 cfu/ml) or R. *nigricans* (1.4x 10^6 cfu/ml) or the consortium of the microorganisms were carefully added and thoroughly mixed. The perforated polythene bags were then tightly sealed. They were incubated at 32^{o}C for periods of time ranging between 0 and 72 h and samples taken at 12 h intervals (0, 12, 24, 36, 48, 60 and 72 h). The unfermented bambara nut (0 h fermentation) served as the control. Samples were taken out for appropriate analyses at regular intervals of 12 h. At the end of each fermentation period, samples were taken and blanched for 20 min and then sliced into smaller units. The slices were drained and dried in an oven that was maintained at 55^{o}C for 24 h, cooled, milled and then sieved with 500 µm sieves to produce fermented bambara nut flour. The flour was packed in polythene bags, sealed and kept in a deep freezer until required for analyses (Olanipekun *et al*. 2015).

Analyses of Vitamins

Vitamin A, vitamin B_2 (riboflavin) and niacin contents were determined using the procedure of AOAC (2005) while thiamine was determined according to the method of Okwu and Josiah (2006).

Determination of Mineral Contents

Minerals (Ca, K, Mg and Fe) were analyzed using the dry-ashing technique according to AOAC (2005) while total phosphorus in each sample was determined spectrophotometrically by the Phospho-Vanadomolybdate method (AOAC, 2005).

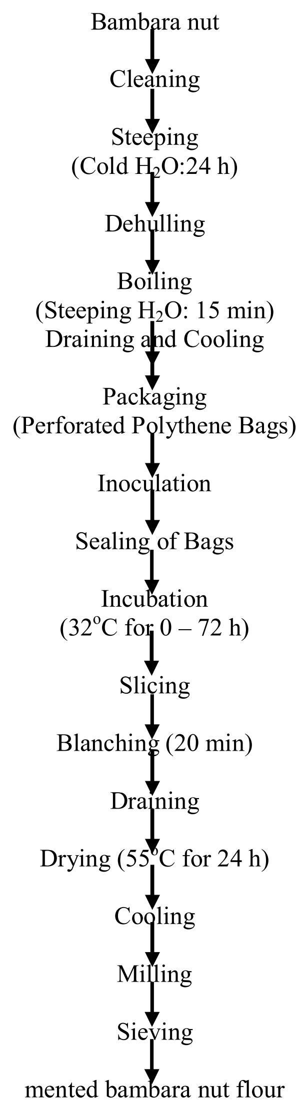


Figure 1: Flowchart for the production of fermented bambara flour
Source: Olanipekun *et al*. (2015)

RESULTS AND DISCUSSION

Vitamin Contents of Fermented Bambara Nut Flour

The influence of fermentation time on the vitamin contents of bambara nut flour are as shown in Figs.1- 4. It was observed that increase in the period of fermentation increased the vitamin levels. Vitamin A, riboflavin and niacin contents were found to increase significantly at $p<0.05$ whereas decreasing trend was observed for thiamine content. Vitamin A increased as shown in Fig.1 ranging from 0.40 - 0.82, 0.38 - 0.57, 0.39 - 0.55, 0.41 -0.85, 0.42 - 0.86, 0.41 - 0.60 and 0.43 - 0.70 mg/100g when *R. oligosporus, R. oryzae, R. nigricans, R. oligosporus* and *R. oryzae, R.* oligosporus and *R. nigricans, R. oryzae* and *R. nigricans, R. oligosporus, R. oryzae* and *R. nigricans*, were used respectively. Slight increases were observed within 0-72 h of fermentation period. Fermenting bambara nut at 0 h however gave the least value while the highest value was obtained at 72 h. The highest value was obtained using the combination of *R. oligosporus* and *R. nigricans* while the least value was observed when *R. nigricans* was used alone.

Also, riboflavin content increased as shown in Fig. 2 from 0.18 - 2.64, 0.19 - 2.25, 0.17 - 2.11, 0.16 - 3.08, 0.18 - 2.96, 0.19 - 2.72 and 0.18 - 3.53 mg/100g and niacin content of fermented bambara nut flour increased as shown in Fig.3 from 0.52 - 3.74, 0.50 - 3.21, 0.52 - 2.99, 0.51- 4.02, 0.53 - 3.98, 0.54 - 3.61 and 0.55 - 4.52 mg/100g using *R. oligosporus, R. oryzae, R. nigricans, R. oligosporus* and *R. oryzae, R. oligosporus* and *R. nigricans, R. oryzae* and *R. nigricans, R. oligosporus, R. oryzae* and *R. nigricans*, respectively. Increasing trends were observed for riboflavin and niacin contents within 0-72 h of fermentation period. The highest values were obtained using the combination of *Rhizopus oligosporus, R. oryzae* and *R. nigricans* while the least valuesobtained byt he singly use of *R. nigricans* for both riboflavin an dniacin contents, respectively. Fermenting bambar a nut flour at 0 h however gave the least while the highest value was obtained at 72 h for both riboflavin and niacin contents.

Decreasing trend was observed for thiamine content of fermented bambara nut flour as shown in Fig. 4. The values ranged from 0.22 - 0.04, 0.20 - 0.05, 0.20 - 0.04, 0.22 -0.02, 0.21 - 0.03, 0.23 - 0.03 and 0.24 - 0.02 mg/100g using *R. oligosporus, R. oryzae, R. nigricans, R. oligosporus* and *R. oryzae, R. oligosporus* and *R. nigricans, R. oryzae* and *R. nigricans, R. oligosporus, R. oryzae* and *R. nigricans*, respectively. Slight decreases were observed within 0-72 h of fermentation period. Fermenting bambara nut at 0 h however produced the highest value while the least value was obtained at 72 h. The least value was obtained using the combinations of *R. oligosporus* and *R. oryzae*, *R. oligosporus*, *R. oryzae* and *R. nigricans* and the highest value by using *R. oryzae.*

Steinkraus *et al. (*1992) had earlier reported that vitamin content of fermented soybean was higher than that of the starting unfermented soybeans in certain cases and lower value in others. Riboflavin content specifically doubled while niacin increased seven times as fermentation period increased. The growth of the bacterium *Klebisella pneumonia* helped in the production of some essential vitamins in vegetarian diets (Steinkraus, 1986). Thiamine content decreased in this study and this is in agreement with the earlier observation of Prinyawiwatkul *et al.* (1997) during related studies on cowpea. The finding is comparable with what had been documented by Waters *et al. (*1993). Similarly, fermentation of the bambara nut with different *Rhizopus* species showed that there was significant reduction in thiamine content of the fermented bambara nut flour . The decrease in thiamine content may be as a result of rapid utilization of thiamine for growth including other functions at a higher rate than its synthesis by fermenting organism. The trend of this finding is similar to the thiamine content of rice which was fermented with *R.oligosporus* as reported by Chavan and Kadam (1989). In the contrary, Philips *et al.* (1983) reported otherwise for the thiamine content of fermented cowpea. Fadahunsi (2009) reported a decrease in thiamine content of fermented bambara nut.

Riboflavin content increased throughout the fermentation period. Philips *et al.* (1983) reported a similar result during the fermentation of cowpea. Contrary trend was observed in riboflavin content when cowpea was fermented for 24 h (Fadahunsi, 2009). The increase in niacin content of bambara nut corroborates the submission of Fadahunsi (2009) though with the exception of thiamine which may be probably due to synthesis of these vitamins by mould. Robinson and Kao (1978) found that water soluble vitamins, except thiamine of horse beans, chick peas and soybeans were greatly increased after fermentation with Rhizopus species which showed that *R.oligosporus* can synthesize both niacin and riboflavin. The mould might be able to synthesize thiamine, but the rate of synthesis is so slow that the organism utilizes readily available thiamine for maximum growth or other functions. Zamora and Fields (1979) reported an increase of 18% riboflavin during a natural lactic acid fermentation of cowpea, but decreased in thiamine content. Vitamins act especially as co-enzymes and precursors of co-enzymes in the regulation of metabolic processes but are known not to provide energy (Keuth and Bispring, 1993).

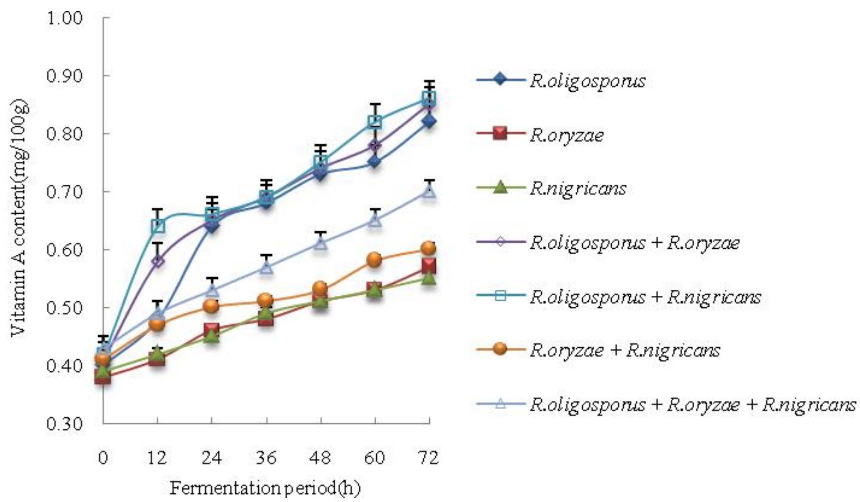


Figure 1: Effect of fermentation on the Vitamin A content of bambara nut flour

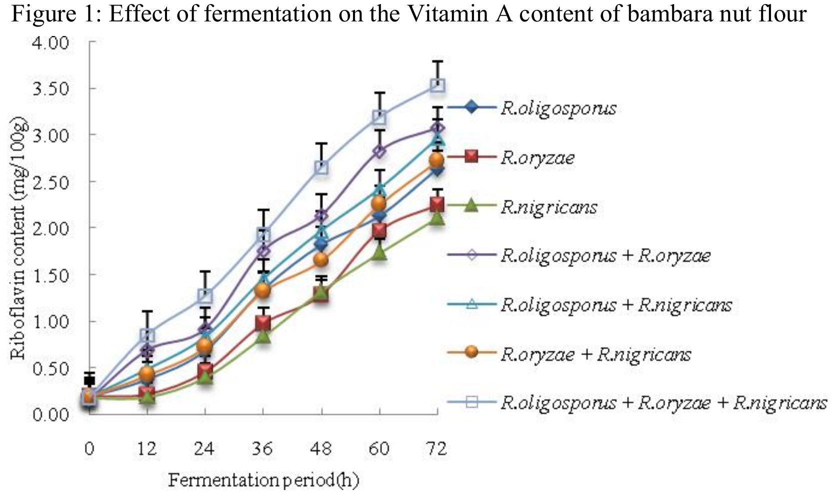


Figure 2: Effect of fermentation on the Riboflavin content of bambara nut flour

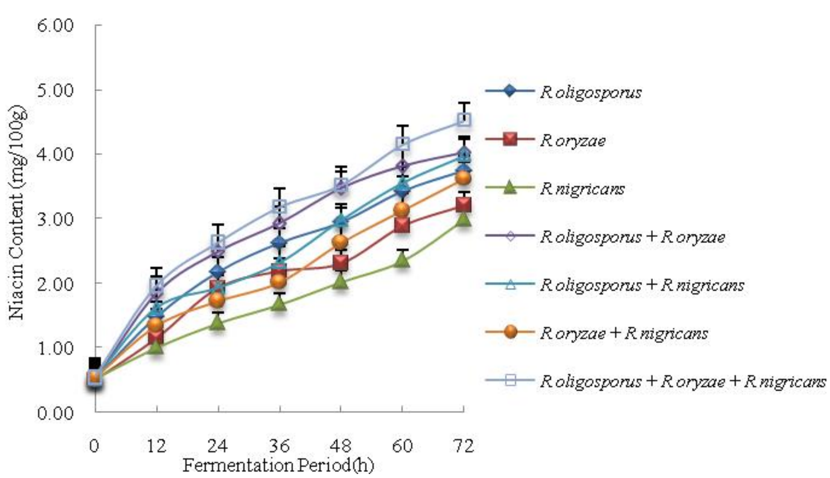


Figure 3: Effect of fermentation on the niacin content of bambara nut flour

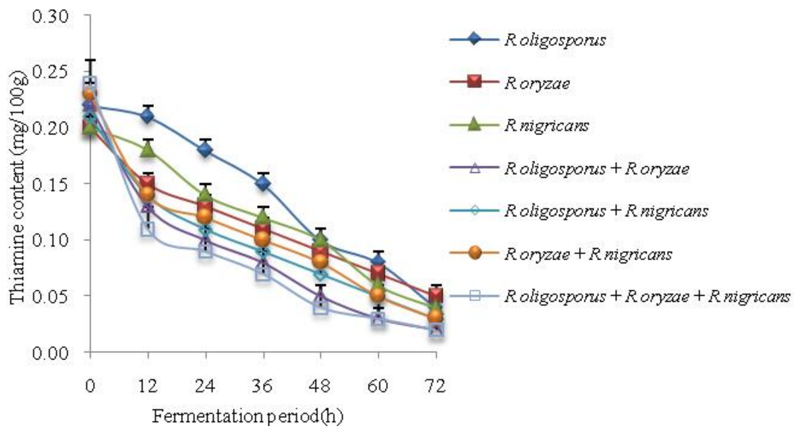


Figure 4: Effect of fermentation on the Thiamine content of bambara nut flour

Mineral Contents of Fermented Bambara Nut Flour

The mineral contents of fermented bambara nut flour are as shown in Figs. 5 - 9. Increasing trends were observed for potassium, calcium, phosphorus, iron and magnesium contents of fermented bambara nut flour.

Calcium Content

The values of calcium content of fermented bambara nut flour as shown in Fig. 5 increased as 0.096-0.235, 0.095-0.211, 0.093-0.196, 0.098-0.2890, 0.095-0.270, 0.094-0.227 and 0.099-0.300 mg/100g by using *R. oligosporus, R. oryzae, R. nigricans, R. oligosporus* and *R. oryzae, R. oligosporus* and *R. nigricans, R. oryzae* and *R. nigricans, R. oligosporus, R. oryzae* and *R. nigricans*, respectively. Slight increases were observed within 0-72 h of fermentation period. Bambara nut fermented with the combination of *R. oligosporus, R. oryzae* and *R. nigricans* had the highest value of 0.099-0.300 mg/100g whereas bambara nut fermented using *R. nigricans* had the least value of 0.093-0.196 mg/100g. Fermenting bambara nut at 0 h however gave the least value while the highest value was obtained at 72 h. The calcium content of fermented bambara nut flour was low when compared with the sunflower seeds (800-1000 mg/kg) by Robinson (1975) and 6010mg/kg was reported for benniseed (Oshodi *et al*., 1995). High amount of calcium, potassium and magnesium have been reported to reduce blood in human (Ranhotra *et al.,* 1998). Increase in calcium contents were obtained in both fermented sorghum and finger millet (Makokha *et al*., 2002). Increase in concentration of calcium content of fermented product (fufu) was previously observed and reported by Oyewole and Odunfa (1989).

Potassium Content

Increasing trends were observed for the potassium content of fermented bambara nut flour as shown in Fig. 6. The values ranged as 1.230 - 1.435, 1.227 - 1.428, 1.228 -1.423, 1.231- 1.453, 1.232 - 1.442, 1.229 - 1.437 and 1.233 - 1.633 mg/100g using *R.oligosporus, R. oryzae, R. nigricans, R. oligosporus* and *R. oryzae, R. oligosporus* and *R. nigricans, R. oryzae* and *R. nigricans, R. oligosporus, R. oryzae* and *R. nigricans*, respectively. Slight increases were observed within 0-72 h of fermentation period.

Fermenting bambara nut at 0 h however gave the least value while the highest value was obtained at 72 h. Bambara nut fermented with the combination of *R. oligosporus, R. oryzae* and *R. nigricans* had the highest value of 1.233 -1.633 mg/100g whereas bambara nut fermented using *R. nigricans* had the least value of 1.228-1.423 mg/100g.

The potassium content of fermented bambara nut was lower than 14.1 mg/100g value obtained from fermented red kidney bean as reported by Audu and Aremu (2011). Slight differences were observed for all the fermented bambara nut flour. Potassium is required for the maintainance of osmotic balance of the body fluids, the pH of the body which assists to regulate muscles and nerves irritability, control glucose absorption and enhance normal retention of protein during growth (NRC, 1989). The sodium to potassium ratio in the body is of great concern for the prevention of high blood pressure. The Na/K ratio of less than one is recommended. Olaofe and Sanni (1998) reported that potassium is the most abundant mineral in agricultural products.

Phosphorus

The phosphorus content of fermented bambara nut flour increased slightly as shown in Fig. 7. Phosphorus values ranged as 0.300 – 0.430, 0.298 - 0.403, 0.297 - 0.398, 0.300- 0.439, 0.299 – 0.432, 0.297- 0.430 and 0.301-0.465mg/100g for using *R. oligosporus, R. oryzae, R. nigricans, R. oligosporus* and *R. oryzae, R. oligosporus* and *R. nigricans, R. oryzae* and *R. nigricans, R. oligosporus, R. oryzae* and *R. nigricans,* respectively. . Slight increases were observed within 0-72 h of fermentation period. Fermenting bambara nut at 0h however gave the least value while the highest value was obtained at 72 h. Bambara nut fermented with the combination of *R. oligosporus, R. oryzae* and *R. nigricans* had the highest value of 0.301 - 0.465 mg/100g whereas bambara nut fermented using *R. nigricans* had the least value of 0.297-0.398 mg/100g.

Phosphorus is always found with calcium in the body, both contributing to the blood formation and supportive structure of the body (Ogunlade *et al.,* 2005). Modern foods rich in animal protein and phosphorus can promote the loss of calcium through urine (Shills and Young, 1992). This led to the concept of calcium and phosphorus ratio. If the Ca/P ratio is low, calcium will be low and there will be high phosphorus intake which leads to calcium loss in the urine more than normal. If the Ca/P ratio is above two, it helps to increase the absorption of calcium in the small intestine. The results of Ca/P ratio in the fermented samples was less than 0.5 which means that fermented bambara nut is a poor source of phosphorus. Increasing level of phosphorus was observed during the fermentation of sprouted hungry rice (acha) which is expected as a result of increased activity of the enzyme and phytase during fermentation. This enzyme hydrolyses the bond between protein-enzyme and mineral to free more phosphorus (Nnam, 2001). Gabriel-Ajobiewe (2011) also reported a significant increase in magnesium, sodium, iron and potassium contents during fermentation of *Canavalia ensiformis(L).* A contrary trend was observed for sweet potato after fermentation (Atum, 2003).

Iron

The values of the iron content of fermented bambara nut flour is as shown in Fig. 8 which ranged as 0.014 - 0.026, 0.013 - 0.023, 0.013 - 0.022, 0.015 - 0.027, 0.014 - 0.025, 0.014 - 0.024 and 0.016 - 0.028 mg/100g using *R. oligosporus, R. oryzae, R. nigricans, R. oligosporus* and *R. oryzae, R. oligosporus* and *R. nigricans, R. oryzae* and *R. nigricans, R. oligosporus, R. oryzae* and *R. nigricans*, respectively. Slight increases wereobserved within 0-72 h of fermentation period. Fermenting bambara nut at 0 h however gave the least value while the highest value was obtained at 72 h. The highest value was obtained using the combination of *R. oligosporus, R. oryzae* and *R. nigricans* and the least value by using *R. nigricans* alone.

Nnam (2000), inclyding Inyang and Zakari (2008) reported increasing trend in the level of iron during fermentation of sprouted hungry rice (acha) and fura, respectively. The report of Elemo *et al. (*2011) was contrary for African locust beans which had 9.3mg/100g of iron. Although, it is not expected that fermentation would increase the mineral content of a product, the hydrolysis of chelating agents such as phytic acid during fermentation improves the bioavailability of minerals (Haard, 1999). This may explain the increases observed in this study. The result corroborated with the report of Latunde-dada (1991) that fermentation of cereals enhanced the availability of elemental iron. The result of this study in term of iron content is lower than the value of 5.9 mg/100g obtained for bambara nut flour (Amarteifio and Moholo (1998).

Magnessium

The magnesium content of fermented bambara nut flour is as shown in Fig. 9. It ranged as 0.018-0.038, 0.017-0.035, 0.017-0.032, 0.019-0.047, 0.020-0.043, 0.019-0.040 and 0.022-0.052mg/100g by using *R. oligosporus, R. oryzae, R. nigricans, R. oligosporus* and *R. oryzae, R. oligosporus* and *R. nigricans, R. oryzae* and *R. nigricans, R. oligosporus, R. oryzae* and *R. nigricans*, respectively. Slight increases were observed within 0-72 h of fermentation period. Fermenting bambara nut at 0 h however gave the least value while the highest value was obtained at 72 h. The highest value was obtained using the combination of *R. oligosporus, R. oryzae* and *R. nigricans* and the least value by using *R. nigricans* alone.

According to Nwokolo (1987), the bioavailability of magnesium was reported to be higher in millet than in sorghum. The values reported for fermented bambara nut is lower than the most abundant ion in plants cells. It is needed for more than 300 different enzyme

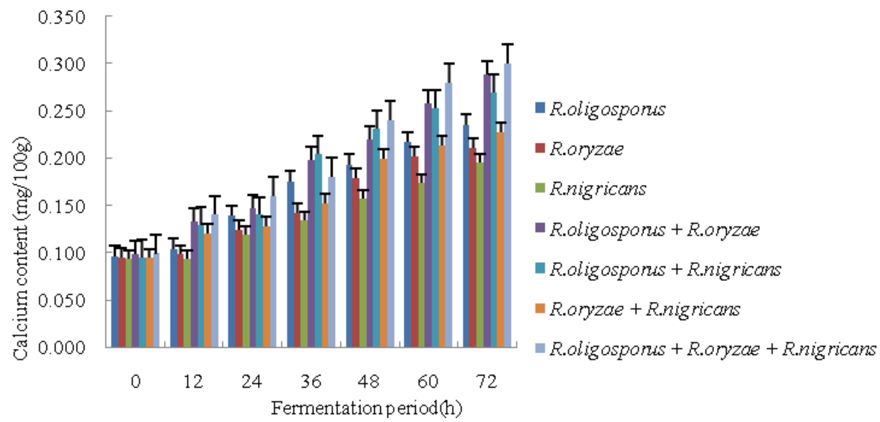


Figure 5: Effect of fermentation on the calcium content of bambara nut flour

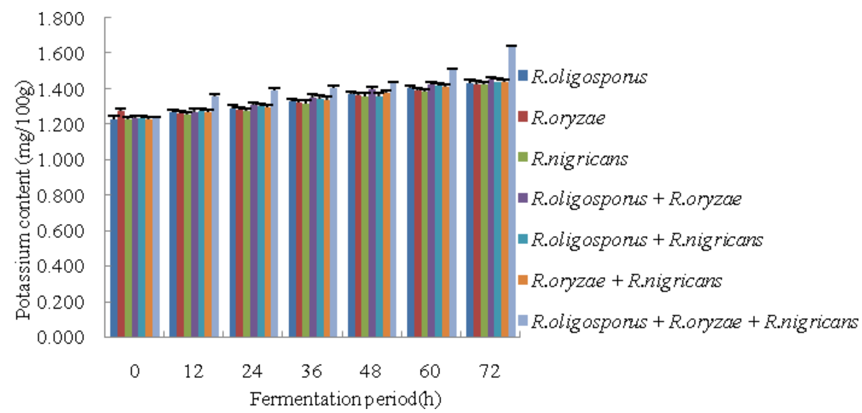


Figure 6: Effect of fermentation on the potassium content of bambara nut flour

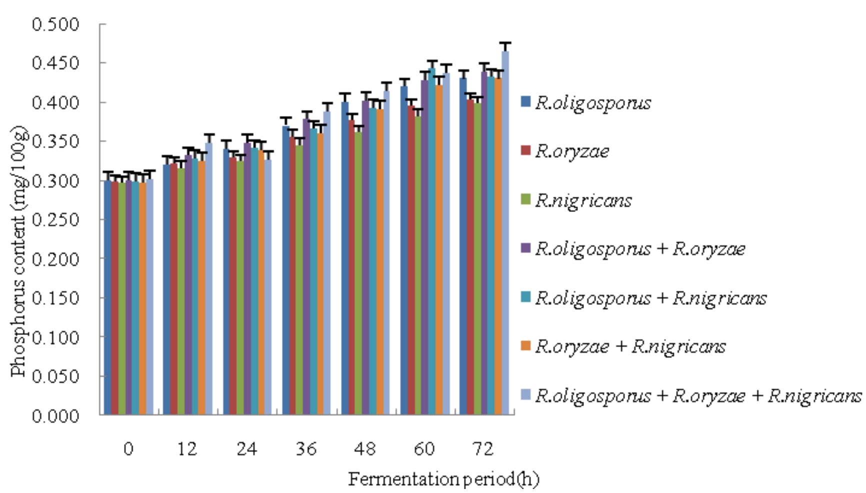


Figure 7: Effect of fermentation on the phosphorus content of bambara nut flour

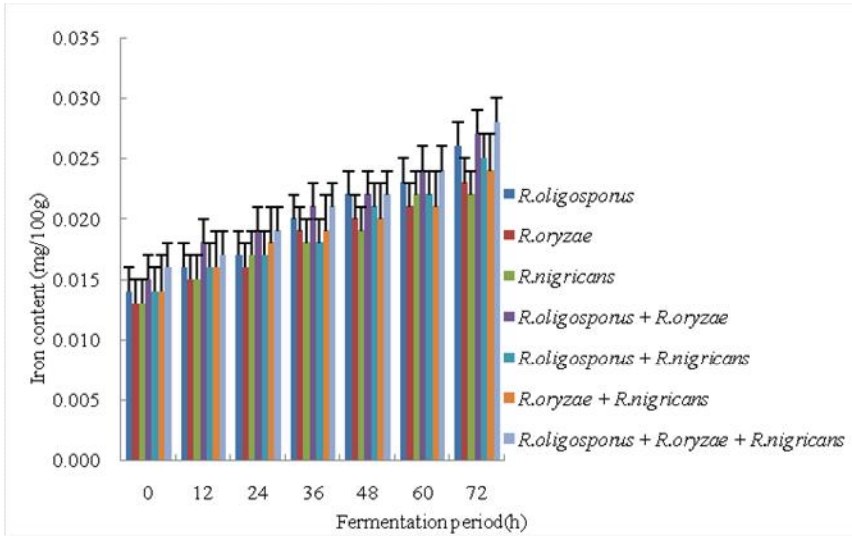
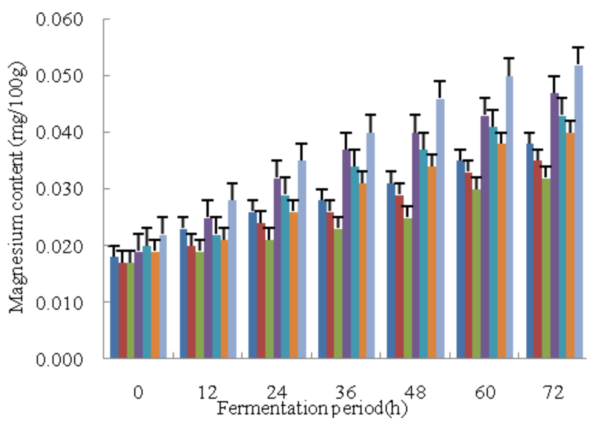


Figure 8: Effect of fermentation on the iron content of bambara nut flour



systems in the body. It is also important in the formation of adenosine trio phosphate, storage of carbohydrate, fat and protein, and including nerve and muscle activity (Aremu *et al.,* 2010).

CONCLUSION

The influence of using different Rhizopus species and their combinations on the mineral and vitamin contents of bambara nut was investigated. Increasing trends were observed in the values of vitamin A, riboflavin and niacin as 21.70 – 66.14%, 0.38 – 0.86 mg/g, 0.16 – 3.53 mg/g, 0.50 – 4.52 mg/g excluding the thiamine which decreased in value as 0.24 – 0.02mg/g, respectively. However, mineral contents (potassium, calcium, phosphorus, iron and magnessium) also increased. Therefore, fermented bambara nut could be used to supplement starchy foods.

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