

Effect of Fermentation on Antinutritional Factors and *in Vitro* Protein Digestibility of Bambara Nut (*Voandzeia subterranean* L.)

Olanipekun, B. F. (Corresponding author)

Department of Food Science and Engineering, Ladoko Akintola University of Technology, P.M.B. 4000, Ogbomoso, Nigeria.

E-mail: bfolanipekun@lautech.edu.ng

Otunola, E. T. and Oyelade, O. J.

Department of Food Science and Engineering, Ladoko Akintola University of Technology, P.M.B. 4000, Ogbomoso, Nigeria.

Abstract

Bambara nut (*Voandzeia subterranean* L.) has better nutritive values than most other legumes. However, it is being underutilized due to long cooking time, antinutritional constituents and dehulling constraints. Fermentation as a unit operation has been able to address most of the factors responsible for the underutilization of some legumes. In this study, a full factorial block design comprising of time (0, 12, 24, 36, 48, 60 and 72 h) and *Rhizopus* combinations (A, B, C, AB, AC, BC and ABC) were used. Three species of *Rhizopus* (*R. oligosporus* (A), *R. oryzae* (B) and *R. nigricans* (C)) and their combinations were used in the fermentation for 3 days. Fermented samples that were collected at 12 hourly intervals were blanched, dried and milled to Bambara Flour (BF). The BF was evaluated for tannin, oxalate, phytate, trypsin inhibitors and *in vitro* protein digestibility (IVPD). The results show that fermentation significantly ($p < 0.05$) reduced the antinutritional factors and enhanced IVPD of bambara nut. Ranges of values for antinutritional factors were tannin (0.35 – 0.02), oxalate (1.54 – 0.39), phytate (35.20 – 10.70) and trypsin inhibitor (3.22 – 0.49mg/g) while *in vitro* protein digestibility were 21.70 – 66.14%. Therefore, fermented bambara nut flour could be useful to supplement starchy foods.

Key words: Antinutritional factors, *in vitro* protein digestibility, *Rhizopus*, bambara flour

1. Introduction

Traditionally, cereals, legumes and other carbohydrate foods play an important role in African diets. Also, researchers had worked towards developing low-income protein foods of plant origin especially for low-income groups in developing countries (Nnam, 2001., Sanni *et al.*, 2005). In achieving this, most traditional foods have been enriched using soybeans as the source of protein towards alleviating the associated low protein problems. Another approach to solving the problem of low intake of protein is to develop high proteinous foods from other crop sources and such crops which are important in tropical climate.

Bambara nut (*Voandzeia subterranean* L. Thouars) belongs to the family of Leguminosae and it is widely grown in Nigeria which is an important tropical country in Africa because it can thrive well on soils too poor for groundnut (*Arachis hypogea*) (Poulter, 1981). The crops can also tolerate drought. It is grown mainly for its seeds which are used as part of food. It plays a role in traditional ceremonies and in gift exchanges (Hepper, 1970; Oloyede *et al.*, 2010). Brough *et al.*, (2001) reported that bambara nut seed makes a balance food as it contains sufficient quantities of carbohydrate, protein and fats with relatively high proportions of lysine and methionine as percentage of the protein.

Despite the protein quality in leguminous seeds, it does not however reach the same level as in animal products. This is due to unbalanced amino acids, presence of antinutritional factors and low digestibility of protein. Processing methods, such as soaking, cooking or fermentation can improve the quality of legume protein (Habiba, 2001., Adebowale and Maliki 2011). During fermentation, microflora may produce proteolytic enzymes which may be responsible for the increase in protein digestibility (Hesseltine, 1983). Also, the elimination of phytic acid contributes to the improvement in protein digestibility of fermented products (Khertarpaul and Chauchan, 1991). Therefore, this research considered the effect of fermentation on the antinutritional factors and *in vitro* protein digestibility of bambara nut.

2. 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, Ladoko Akintola University of Technology, Ogbomoso, Nigeria and the Institute of

Agricultural Research and Training (IAR&T), Ibadan. The chemicals that were used were obtained from May and Baker Company, England and Sigma Chemical Company Limited, U.K. and were of analytical grade.

2.1 Sample preparation

2.1.1 Subculturing of fungal cultures

The organisms used for fermentation namely; *R. oligosporus*, *R. oryzae* and *R. nigricans* were subcultured by the procedure described by Fadahunsi (2009): Five hundred millilitres of PDA was prepared by using 18 g of potato dextrose agar and 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 30°C for 4 days.

2.1.2 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 combinations within 0 -72h fermentation period.

2.1.3 Preparation of fermented bambara flour

Bambara nut was fermented according to the method of Fadahunsi (2009). 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 dry a little at 30°C 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* (2.0×10^6 cfu/ml) , *R. oryzae* (1.7×10^6 cfu/ml) or *R. nigricans* (1.4×10^6 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°C for periods of time ranging between 0 and 72 h 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°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 (Fadahunsi, 2009).

2.2 Determination of the contents of anti-nutritional factors

Phytic acid and trypsin inhibitors were determined by AOAC (2005) while the tannic and oxalic acids were determined using the procedure of Medoua *et al.* (2007).

2.3 In vitro protein digestibility of fermented bambara flour

In vitro protein digestibility of the fermented bambara nut flour sample was evaluated according to the method of Chavan *et al.* (2001).

2.4 Statistical analysis

All data were obtained in three replicates and were subjected to statistical analysis using the Statistical Analysis Software (SAS) Version 9.1. The Analysis of Variance (ANOVA) was used to compare the results while Duncan Multiple Range was used for their means separation (Duncan, 1955; Poste *et al.*, 1991).

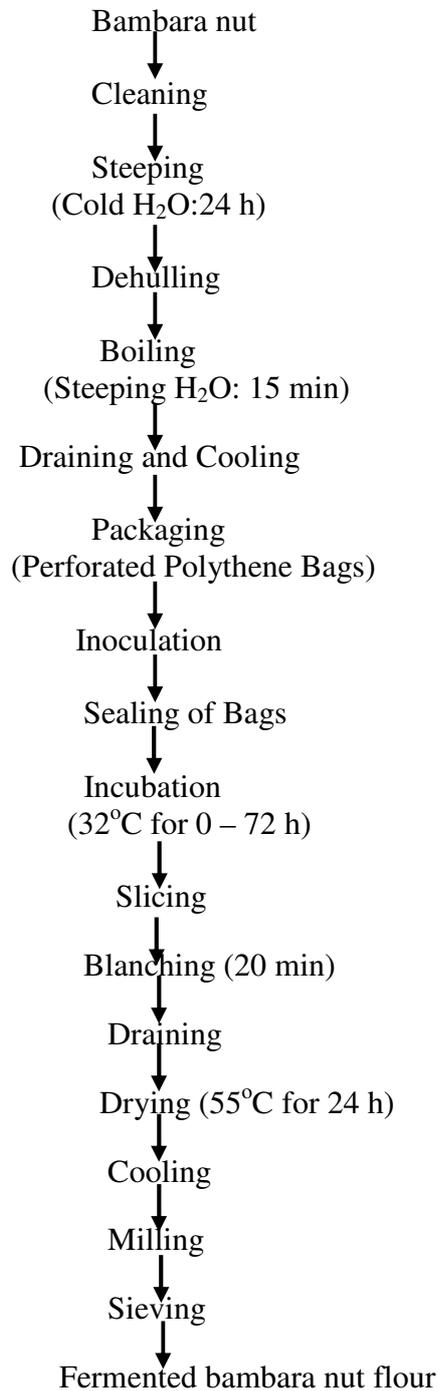


Figure 1: Flowchart for the production of fermented bambara flour
Source: Fadahunsi (2009)

3. Results and discussion

3.1 Effect of fermentation on level of antinutritional factors of bambara flour

3.1.1 Tannin content

The results of the antinutritional factors of fermented bambara flour are shown in Figs. 2 - 5. The tannin content decreased within 0-72h fermentation period ranging from 0.33-0.10, 0.34-0.12, 0.35-0.15, 0.33-0.06, 0.31-0.07, 0.32-0.08 and 0.31-0.02 mg/g with *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 gave the highest values while the least values were obtained at 72 h. The highest value was obtained using *R. nigricans* and the least value by combination of *R. oligosporus*, *R. oryzae* and *R. nigricans*.

Reduction in tannin due to the cooking and fermentation might have been caused by the activity of polyphenol oxidase or fermented microflora on tannins (Reddy and Pierson, 1994). Boiling was the most effective processing technique that reduced the tannin content of the seed flours. Reddy and Pierson (1994) reported that dehulling and cooking eliminated more than 90% of the tannin content in soybean due to their predominance in seed coats, while dehulling, cooking and fermentation were generally reported to reduce tannin content of cereals and other foods (Rehman and Salariya, 2005., Jude *et al.*, 2009).

The findings agreed with the observation of Fagbemi *et al.* (2005) on breadnut, cashew nut and fluted pumpkin. Also, reduction of tannin content on fermented papaya seed was observed by Afolabi *et al.* (2011). The observed decrease in tannin with increase in fermentation time agrees with the report of Onweluzo and Nwabugwu (2009). The tannin content of fermented bambara nut flour decreases from 0.33 to 0.02 mg/100g within the period of fermentation which was lower in value compared to 0.16mg/100g reported by Abiodun and Adepeju (2011) on dehulled bambara nut flour.

3.1.2 Oxalate content

The oxalate content of fermented bambara flour is as shown in Fig. 3. The oxalate content decreased within 0-72h fermentation period as 1.51-0.75, 1.53-0.81, 1.54-0.85, 1.50-0.53, 1.51-0.64, 1.51-0.70 and 1.50-0.39 mg/g for *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 gave the highest value while the least value was obtained at 72 h. The highest value was obtained using *R. nigricans* and the lowest value by the combination of *R. oligosporus*, *R. oryzae* and *R. nigricans*. Oxalate content of fermented bambara nut flour showed a decreasing trend within 0-72h fermentation period. Slight differences were observed in the fermented samples. Similar results that showed reducing oxalate contents were observed for some commonly consumed food products such as cocoyam and yellow yam (Adeniyi *et al.*, 2009., Afolabi *et al.*, 2011). Iwuoha and Kalu (1995) reported 82.1% and 61.9% oxalate reduction in cocoyam flour obtained from boiled and roasted cocoyam tubers, respectively. Also, reduction of oxalate was observed in cocoyam flour and dehulled bambara nut flour (Abiodun and Adepeju, 2011., Oke and Bolarinwa 2012). Albihn and Savage (2001) reported that boiling causes considerable skin (epidermal) ruptures and facilitates the leakage of soluble oxalate into cooking water.

3.1.3 Phytate content

Phytate content of fermented bambara flour result is shown in Fig. 4. The values obtained showed a decreasing trend from 34.90 - 13.80, 35.20 - 14.00, 34.80 - 15.70, 35.00 - 12.00, 35.10 - 12.80, 34.90 - 13.50, 35.20 - 10.70 mg/g with *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. Significant decreases were observed within 0-72 h of fermentation period. Fermenting bambara nut at 0 h however gave the highest value while the least value was obtained at 72 h. The highest value was obtained using *R. nigricans* with the least value by combination of *R. oligosporus*, *R. oryzae* and *R. nigricans*.

Polyphenolic compounds can interact with proteins and reduce their digestibility, as well as alter amino acid availability and functional properties (Reddy and Salunkhe, 1981). However, they may also be beneficial due to their strong antioxidant activity (Mathaus, 2002). The result of this finding agrees with the report of Amoa and Muller (1976) of 31.1% reduction in phytic acid content for kenkey (fermented maize) and 45.5% reduction reported by Sudarmadji and Markakis (1977) on the fermentation of common beans to tempeh. It is also consistent with the results of Fardiaz and Markakis (1981) and Sutardi and Buckle (1985) who reported 48 - 96.3% and 54.77% reduction in phytic content of peanut and soybean respectively. Processing, especially

fermentation, has been reported to reduce phytic acid content of cereals, legumes and tubers as a result of the activity of the endogenous phytases from both raw ingredient and inherent microorganisms which hydrolyse phytic acid in many fermented food preparations into inositol and orthophosphate (Reddy and Pierson, 1994; Sandberg and Andlid, 2002).

3.1.4 Trypsin inhibitor content

Decreasing trend with *Rhizopus* species was observed for trypsin inhibitor of fermented bambara flour as shown in Fig.5. The values varying as 3.22 - 0.66, 3.20 - 0.78, 3.21 - 0.94, 3.19 -0.51, 3.20 -0.55, 3.20 - 0.53 and 3.18 - 0.49 mg/g for *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. Significant decreases were observed within 0-72 h of fermentation period. Fermenting bambara nut at 0 h however gave the highest value while the least value was obtained at 72 h. The highest value was obtained using *R. nigricans* and the least value by the combination of *R. oligosporus*, *R. oryzae* and *R. nigricans*.

Fermentation is the most effective processing technique to reduce trypsin inhibitor activity in the seed flours. The results obtained in this work agreed with the observations of the previous workers (Paredes-Lopes and Harry, 1989., Roozen and De Groot 1985) on cooked and fermented soybean, and common beans where reduction in trypsin inhibitor activity is between 91.4-99.9% and 52% were reported for fermented soybean and hot soaked cowpea respectively. Investigation of the parameter for bread nut, cashew nut and fluted pumpkin (Fagbemi *et al.*, 2005) shows similar trend. Previous workers indicated significant reductions in trypsin inhibitor activity content (Mbithi-Mwikya *et al.* 2001; Ojokoh *et al.* 2012) of cereal and legumes. Also, during tempeh fermentation *R. oligosporus* had been reported to reduce or eliminate trypsin inhibitors (Hachmeister and Fungi (1993)., Matueschek *et al.* (2002)).

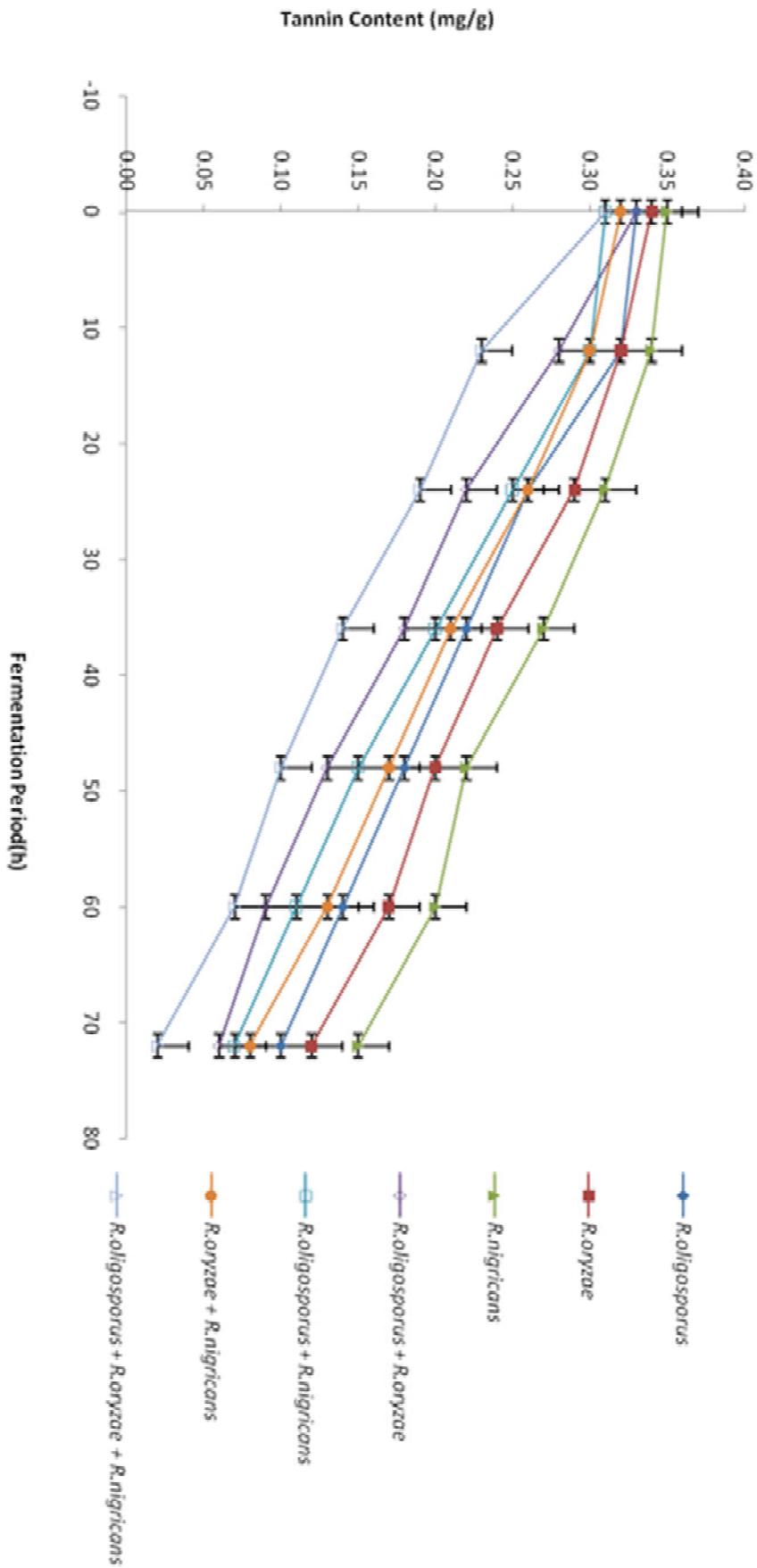


Figure 2: Effect of fermentation on the tannin content of bambara flour

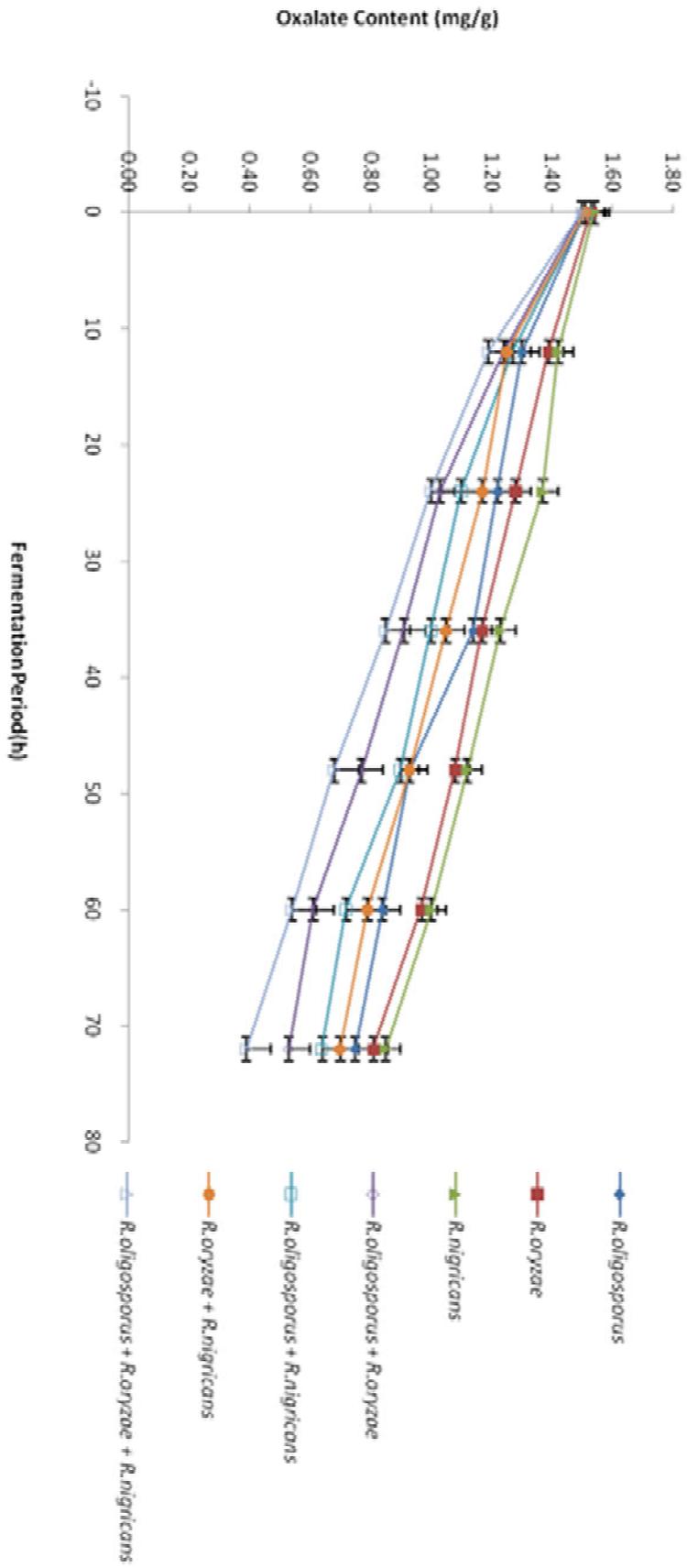


Figure 3: Effect of fermentation on the oxalate content of bambara flour

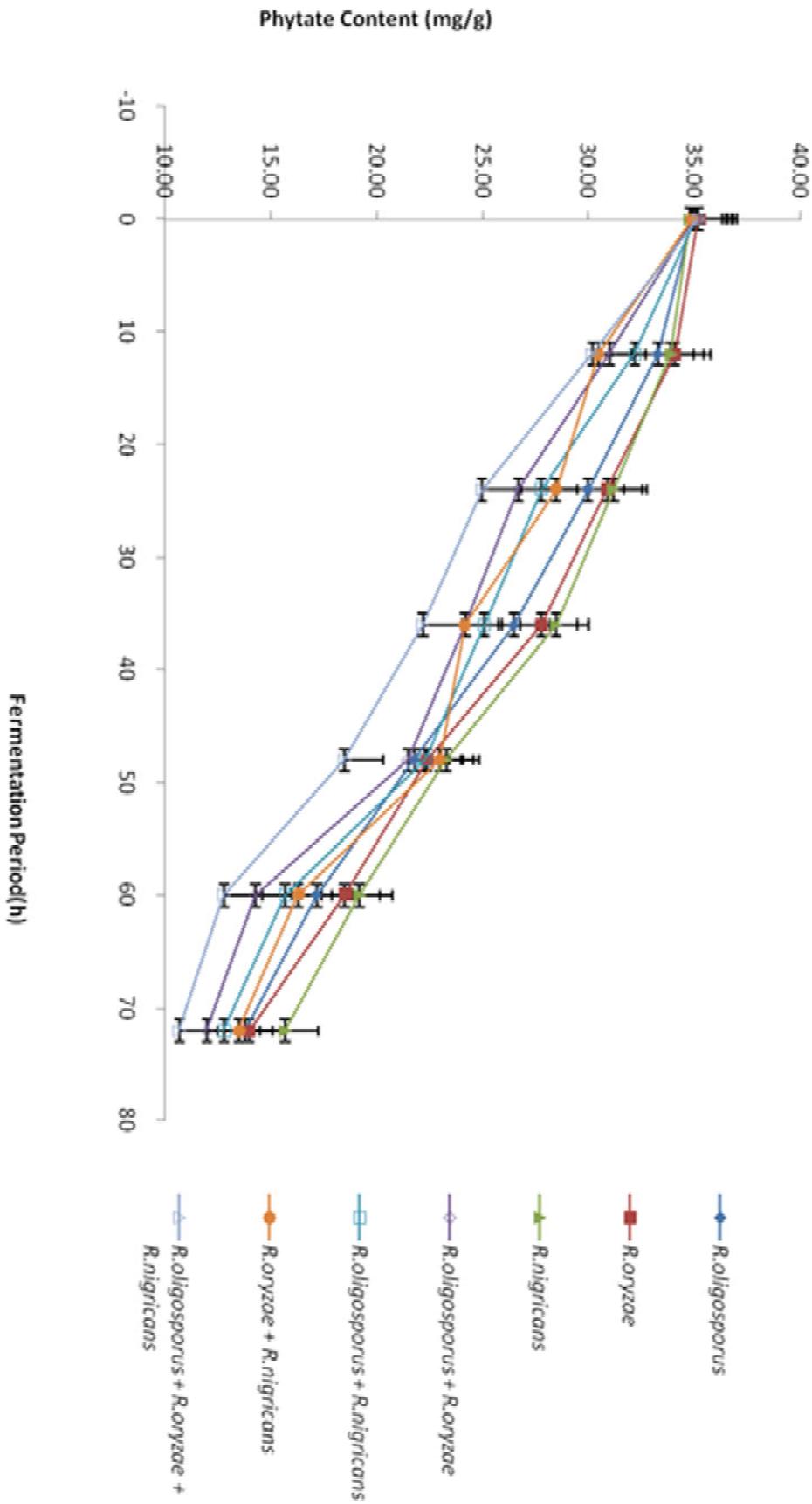


Figure 4: Effect of fermentation on the phytate content of bambara flour

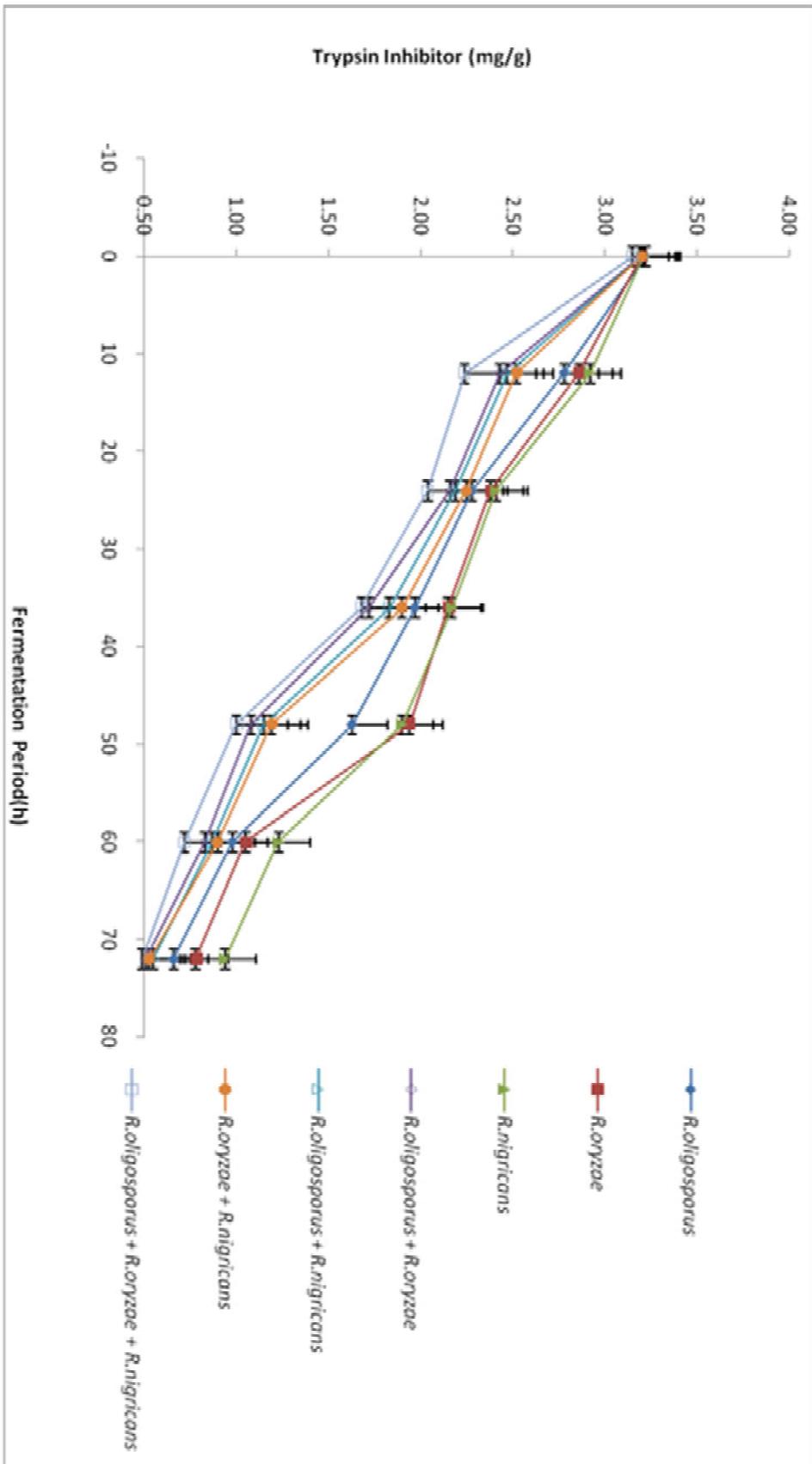


Figure 5: Effect of fermentation on the trypsin inhibitor of bambara flour

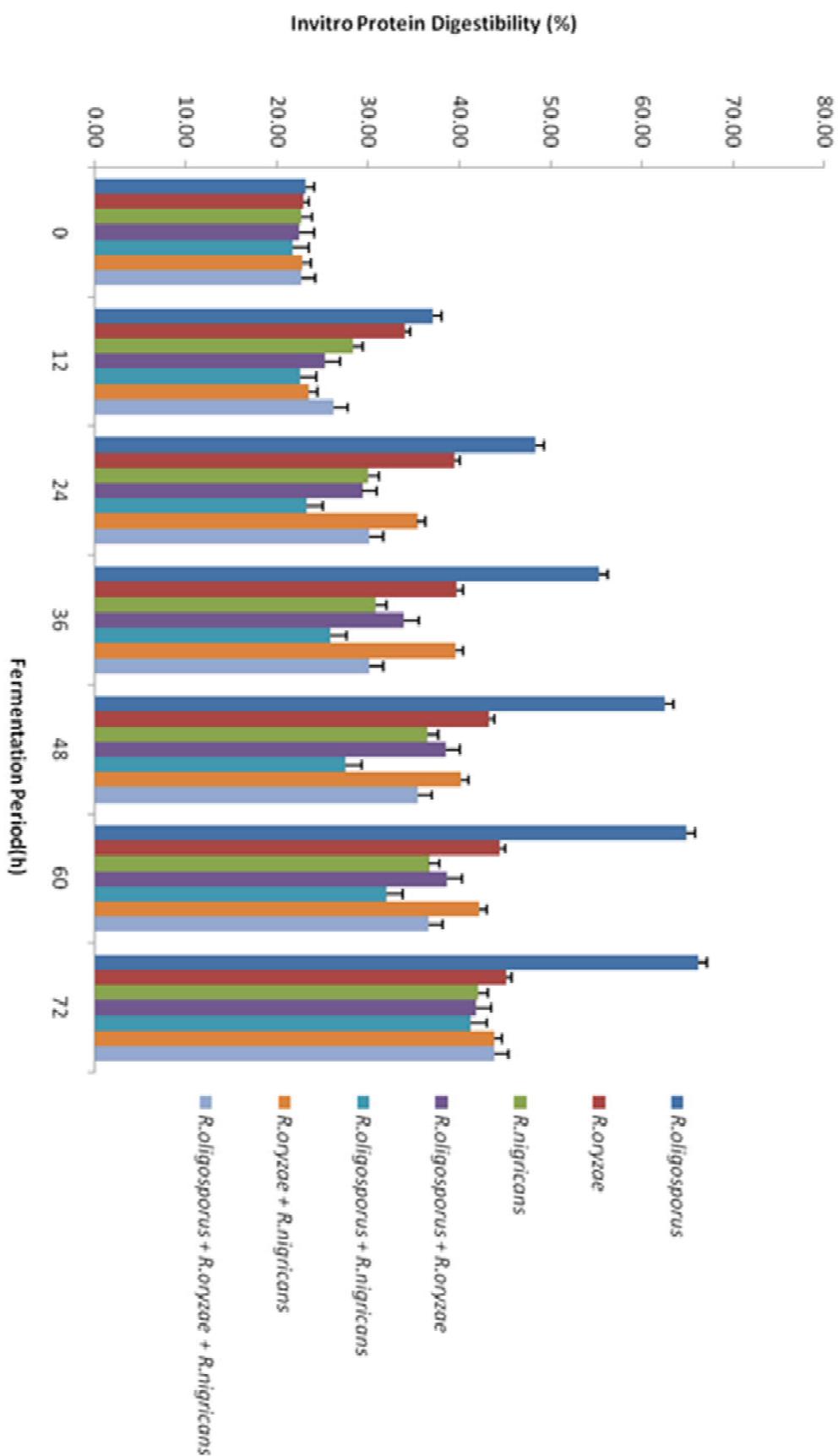


Figure 6: Effect of fermentation on the *in vitro* protein digestibility of bambara flour

3.2 *In-vitro* protein digestibility of fermented bambara flour

Fig. 6 shows the *in vitro* protein digestibility of fermented bambara flour. The percentage *in vitro* protein digestibility increased significantly within 0-72h fermentation period for all the fermented bambara nut flour samples. The *in vitro* protein digestibility increased as 23.07 - 66.14, 22.84 - 45.06, 22.65 - 42.00, 22.38 - 41.80, 21.70 - 41.18, 22.78 - 43.79 and 22.59 - 43.80% for *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. Significant 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 (66.14 %) was obtained using *R. oligosporus* singly and least value (21.70%) was by the combination of *R. oligosporus* and *R. nigricans*.

Kiers *et al.* (2000) reported that digestibility of cereals and legumes increased during cooking and fermentation. Microflora may produce proteolytic enzymes during fermentation which may be responsible for the increase in protein digestibility (Heseltine, 1983). Also, the elimination of phytic acid contributes to the improvement in protein digestibility of fermented millet (Khetarpaul and Chauchan, 1991). The results obtained in this study agrees with Mohiedeen *et al.* (2010) who reported that fermentation is found to improve the *in vitro* protein digestibility of the two maize cultivars and this could be attributed to the partial degradation of complex storage proteins into more simpler and soluble products. It has been reported that the reduction in pH during fermentation plays an important role in enhancing native proteolytic enzymes activity and consequently promotes the breakdown of proteins to smaller polypeptides which are easily digested by enzymes (Khetarpaul and Chauhan, 1989., Yagoub and Abdalla, 2007). Murwan and Ali (2011) reported an increasing trend *in vitro* protein digestibility of Dabar and Tabat as period of fermentation increases.

4. Conclusions

Fermentation process using *Rhizopus* species singly and with their combinations showed reduction in antinutritional factors with values of tannin (0.35 – 0.0249 mg/g), oxalate (1.54 – 0.3949 mg/g), phytate (35.20 – 10.7049 mg/g) and trypsin inhibitor (3.22 – 0.49 mg/g) for bambara nut. The lowest values for all the investigated antinutrients were observed at 72 h of fermentation period when using the combinations of *R. oligosporus*, *R. oryzae* and *R. nigricans*. Therefore, the improved nutritive value of the fermented bambara flour was observed due to increase in value of *in-vitro* protein digestibility with the highest value of 21.70 – 66.14%, obtained with the singly use of *R. oligosporus*.

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