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Research Paper

Anti-nutrient Content, and *in vitro* Protein Digestibility (IVPD) of Infant Food Produced from African Yam Bean (*Sphenostylis sternnocarpa*), and Bambara Groundnuts (*Voandezela subterranean*)

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Abstract. African yam bean (AYB) (*Sphenostylis stenocarpa*) and bambara groundnut (BG) (*Voadzeia subterranean*) are some of the pulses with unutilized high nutritional value. The aim of this study was to assess the anti-nutrient content of raw and processed AYB and BG, and infant weaning diets formulated from flour processed from both crops, using a Nestle Infant formula (Nutrend) as standard. Reduction of anti-nutrient content was carried out using soaking and germination procedures, followed by oven drying at 105^{o}C. After germination there was 92.13% tannin, 82.01% phytate, 60.14% cyanate and 80.36% oxalate reductions in AYB. There was 84.34% tannin, 82.34% phytate, 50.04% cyanate and 82.14% oxalate decreases in BG. Anti-nutrient contents of diets ranged from 14.31±0.75 - 15.20± 0.61 mg/g for tannin, 17.02±0.81 - 24.82±1.65 mg/g for phytate, 1.34±0.01 - 1.43±0.01 mg/g for cyanate and 0.14±0.02 - 0.55±0.05 mg/g for oxalate. These values compared well with those of the standard formula which gave 15.20±0.52 mg/g, 26.01±1.64 mg/g, 1.56±0.03 mg/g and 0.82±0.05 mg/g anti-nutrient contents of tannin, phytate, cyanate and oxalate. There was a significant ($p<0.05$) increase in digestibility values of diet 1 (87.60%), diet 2 (85.65%), and diet 3 (84.35%), while the standard diet 4 gave 85.31% digestibility value. Protein concentration (Pe%) of diets were 25.97%, 24.27% and 22.96%, for diets 1, 2 and 3, compared to 18.03% obtained for standard diet 4. The PE% profile and low anti-nutritional contents in test diets suggest that the diet formulas may serve as alternative infant weaning formulations. Thus, AYB and BG may serve as alternative infant weaning food and bridge in food security gap for weaning formulas.

Keywords: African yam bean, Bambara groundnuts, anti-nutrients, Protein digestibility.

INTRODUCTION

Legumes are foodstuffs with very important nutritional significance waiting to be exploited in Nigeria and other African countries. Though, plant proteins are generally nutritionally inferior to those of animal protein, the combination of plant protetin can provide complete essential amino acids (Nwokolo, 1987; Duke *et al*., 1977; NAS, 1979). African yam beans (*Sphenostylis stenocarpa*) and bambara groundnuts (*Voadzeia subterranean*) are leguminous crops that could help meet protein dietary needs of the ever increasing imbalance of rates of population growth and food production. The imbalance in production and consumption patterns far outstretches available incomes in many societies in the world. Infant weaning formulas are expensive and unaffordable by low income families of sub-Sahara Africa. Bambara groundnut and African yam bean are indigenous plants cultivated as food crops in West Africa and grows particularly in Nigeria, Ghana, Togo, Cote d'ivoire and Cameroon (Porter, 1992 and Ajayi *et al*., 2009). The crops are cultivated in localized areas where they are used as food in adult nutrition. Their utilization for infant weaning purposes has been very low due to lack of awareness (Klu *et al*., 2001, Chinedu and Nwinyi, 2012), compared to other popular legumes like peanuts and soybeans. The nutritional benefits derivable from these legumes is their potential utilization for adult and infant weaning food by reduction of the anti nutrient contents and formulation of infant food and diets (Brough *et al.* 1993, Poulter and Caygill, 2006).

According to Welch and Graham (2004), anti-nutrients can be eliminated from plant derived food materials using genetic engineering; and anti-nutrient compounds may have beneficial effects especially if genetically modified. There are strong claims that some antinutrients such as oxalates and cyanates could make foods more nutritious, but may not improve human health (Igile *et al*., 2013). African yam bean and bambara groundnut are hard seeds, and are not extensively utilized due to their peculiar hard-to-cook nature which prolongs cooking time and impacts negatively on the high cost of cooking fuel. Traditional methods of processing, including soaking in water followed by cooking reduces the concentration of anti-nutrients to tolerable levels. Other simple ways of reducing anti-nutrients in these seeds include preparative procedures, such as fermentation, germination, roasting, soaking in warm water and boiling (Oluwole and Taiwo, 2009). These legumes are also unacceptable to people and often are underutilized due to undesirable qualities like flatulence producing factors and majorly because of the presence of anti-nutritive constituents (Liener, 1994).
The search for alternative and indigenous infant weaning formulas using available food crops is gaining acceptability. Ikpeme *et al.* (2012) had evaluated the nutritional and sensory characteristics of soybean *and tigernut formulated infant food targeted at* rural dwellers with lower income. The study showed that the quality of the infant weaning formula compared favourably well with the standard reference brand, in terms of protein content, mineral and vitamin composition as well as the energy value (Ikpeme *et al*., 2012).

This study therefore aimed at determining the anti-nutritive concentrations and the effects of processing on the anti-nutrient content of African yam bean and bambara groundnut using a combination of soaking and germination method, followed by oven drying technique to speed up the reduction of anti-nutrients in the formulated diets. The success of the use of African yam bean and bambara groundnut for infant weaning purposes would surely boost family incomes since the new formulas would be far less expensive and easily affordable compared to standard brands sold in the market.

MATERIALS AND METHODS

One hundred and fifty grams of each sample of African yam beans (AYB), and bambara groundnut (BG) were purchased from Owerri main market in Imo State of Nigeria. Impurity, stones and seeds damaged by insects were sorted from the legumes, and the samples were washed separately in clean tap water and re-weighed. The seeds were then soaked in tap water for 12 hours at room temperature. The seeds were rewashed in lined basket for 3 days and allowed to germinate during the three-day period. The germinated seeds were washed with water twice to remove adhering husk and microbes everyday, which consequently restricted fermentation of the seeds. At the end of the third day when the mean length of the sprout was about 1cm, the seeds were finally rinsed in clean tap water, oven- dried in a Gallenkamp oven (Model 1H-150) at 85˚C for 14 hours and milled into flour and stored under refrigeration. Fresh carrots were washed with saline solution, grated and oven-dried at about 60˚C for 3 hours and added to the weaning formulation to enhance the vitamin composition of the test diets by enriching the formulas with vitamins B1, B2, Folic acid and Niacin.

Diet Formulation

The various flour samples were oven-dried at 40˚C for 3 hours before being used for diet formulations as shown below in the Table 1.

Determination of Anti-nutrients in Diet Samples

Determination of phytate content in diet samples

Phytate concentration in test diets was determined using the method described by Reddy and Love (1999). This was carried out in four steps described as follows:

(i) Extraction of phytate and conversion to iron-phytate

100 mls of 0.5M HCl was added to 2.0g of each sample. The mixture was swirled for 2 hours and filtered. 10mls aliquot was neutralized with 0.5M NaOH that was made slightly acidic with 0.17M HCl. This was then diluted to 50ml with distil water. To 10mls of this solution, 4mls of 0.25% $FeCl_3$ was added and heated for 15 minutes at 100˚C. Thereafter, it was centrifuged for 10miutes and the supernatant discarded. The residue was first washed with 0.5M HCl and then 0.17 M HCl. It was centrifuged and supernatant discarded.

(ii) Precipitation of ferric hydroxide

The ferric phytate was dispersed in one milliliter (1 ml) of distil water. 3mls of 1.5M NaOH was added and heated for 10 minutes at 100˚C. The mixture was cooled and

Table 1: Diet Formulation

Diet	AYB (g)	BG (g)	Total (g)
1	20	80	100
2	50	50	100
3	80	20	100
4	Standard Infant Food (Nutrend)		100

AYB: African yam beans. **BG:** Bambara groundnut

centrifuged for 15 minutes, and the supernatant discarded. The precipitate was dissolved in 40mls of 3.2 M HNO_3 and made up to 100mls with distilled water.

(iii) Determination of iron in sample

20ml of sample solution was pipetted into 50mls flask. 1 ml of 2M HNO_3 and 5mls potassium thiocyanate were added and diluted to volume. 2 – 10 mls of standard $FeCl_3$ solution was added to the mixture and mixed thoroughly and the absorbance readings taken at 510 nm.

(iv) Determination of phytate

Phytic acid was calculated based on iron to phosphorus ratio of 5:6. Phytate – phosphorus was obtained by multiplying the ratio of the atomic weight of phosphorus (in phytate) and the molecular weight of phytate by the value of phytate obtained. Non- phytate phosphorus was obtained by subtracting the phytate – phosphorus value from total phosphorus of each sample.

Determination of tannin content

Tannin content was estimated using the Folin-Denis spectrophotometric method described by Pearson (1976) as follows; 0.1g of dried flour of each sample was weighed into 100mls flask. 50mls of distil water was added and boiled for one hour. The mixture was filtered into 50mls flask and diluted to volume. Standard tannin (0.1mg/ml) was prepared on the working range 0 – 10mls. 10mls of aliquot were placed into different 50mls flasks. The solution was made up to 35mls before 2.5mls follin-D solution was added. The mixture was shaken and 10mls of 17% Na_2CO_3 added. The mixture was made up to 50mls and allowed to stand for 30 minutes. The absorbance readings were taken at a wavelength of 725nm.

Determination of cyanic acid

Hydrocyanic acid concentration was determined using the alkaline titration method of AOAC (1990) as follows; 100ml distil water was added to 10g of sample in a 500mls distillation flask. This was allowed to stand for 2-4 hours for autolysis to take place. 150ml of distillate was placed in 20mls of 2% NaOH. To 100ml aliquot of distillate was added 8mls of 6M NH_4OH and 2mls of 5% KI mixed and titrated with 0.2N $AgNO_3$. The end-points were turbid against a black background. Titre values were used to calculate cyanide content by gravimetry.

Determination of Oxalic acid

Total oxalate was determined according to the procedure described by Day and Underwood (1986).

Determination of *in vitro* Protein digestibility

0.5g of sample was weighed into 25ml conical flask. To this, 15mls of 0.1M HCl containing 1.5mg pepsin was added. The flasks were shaken for 3 hours in water-bath at 35 ˚C. 4mls of 0.5M NaOH was added to neutralize the acid. The samples were then treated with 7.5mls of 0.2M phosphate buffer (pH 8.0) containing 4.0mg of pancreatin. The mixture was shaken for 24 hours at 35˚C in a water-bath. Sodium ferrocyanide (one ml) was then added to terminate the reaction. The resultant mixture was centrifuged at 3000rpm for 30 minutes. The residue was digested by Kjeldahl method (A.O.A.C., 1984). Protein content was determined by multiplying nitrogen (%) by the factor 6.25. From this, percentage digestibility was calculated as protein in sample minus protein in residue, divided by protein in sample and multiplied by 100, according to the equation.

$$\text{Protein digestibility (\%)} = \frac{\text{protein in sample} - \text{protein in residue}}{} \times \frac{100}{1}$$

Statistical analysis

All data were expressed as Mean ± S.E.M. from n=3 replications. Data were analyzed using one way analysis of variance (ANOVA) at $p<0.05$ level of significance.

RESULTS

Tables 2, 3 and 4 shows the anti-nutrient content of raw

Table 2: Anti-nutrient content of raw and processed African yam bean and Bambara groundnut samples (mg/100g)

Sample	Tannin	Phytic Acid	Cyanic Acid	Oxalic Acid
Raw AYB	713.50 ±4.15	501.61± 2.52	5.03 ±0.23	2.25 ±0.08
Processed AYB	56.15 ± 2.32	90.23 ±2.71	2.01± 0.12	0.44± 0.05
% decrease	92.13	82.01	60.14	80.36
Raw BG	315.01 ± 2.31	617.01± 5.42	3.51± 0.21	1.96± 0.04
Processed BG	49.32 ±3.11	108.92± 3.23	1.75± 0.10	0.35± 0.07
% decrease	84.34	82.34	50.04	82.14

Mean ± SEM, n=3 **AYB:** African yam beans. **BG:** Bambara groundnut.

Table 3: Anti-nutrient contents of test diets and standard diet (Nutrend) mg/100g.

Samples	Tannin	Phytic Acid	Cyanic acid	Oxalic acid
Diet 1	14.31± 0.75	24.82± 1.65	1.34 ±0.01	0.82 ±0.05
Diet 2	15.01± 0.62	21.78 ±0.43	1.41 ±0.02	0.45 ±0.06
Diet 3	15.20 ±0.61	17.02 ±0.81	1.43 ±0.01	0.55 ±0.05
Diet 4	15.20 ±0.52	26.01 ±1.64	1.56 ±0.03	0.14 ±0.02

Mean **±** SEM, n =3

Table 4: *In-vitro* digestibility and protein concentration (Pe%) of test diets

Samples	Protein Digestibility (%)	Protein Concentration (Pe%)
Diet 1	87.36	22.96
Diet 2	85.65	24.27
Diet 3	84.35	25.97
Diet 4	85.31	18.03

and processed African yam bean and bambara groundnut samples; anti-nutrient content of test diets and the standard diet (Nutrend); and the *In-vitro* digestibility and protein concentration (Pe%) of test diets respectively.

The results in Table 2, showed that there is significant ($p<0.05$) decrease in the tannin level in the African yam bean and bambara groundnut by 92.13% and 84.34% respectively after processing them. There was also a significant ($p<0.05$) loss of phytic acid in African yam bean and bambara groundnut by 82.01% and 82.34% respectively. The phytate content of AYB was found to be lower than that of BG. Cyanic acid content of AYB and BG was reduced by 60.14% and 50.04% respectively (Table 2).

There was also a significant ($p<0.05$) reduction in the tannin content of the test diets (Table 3). The tannin contents were reduced to levels which compared favourably with those of nutrend (Standard diet (Table 4). Tannin content of Diet 1 (14.31 ±0.75 mg/g), Diet 2 (15.01± 0.52 mg/g), and Diet 3 (15.20 ±0.61 mg/g) were low, and similar to the result for Nutrend (15.20 ± 0.52 mg/g).

Results of phytate content of test diets were significantly ($p<0.05$) lower (17.02 ±0.81 - 24.82± 1.65 mg/g) than the phytic acid content in Nutrend (26.61±1.64 mg/g). All test diets gave lower cyanic acid contents compared to the standard diet (Nutrend).

The values of the in vitro protein digestibility of the test diets and the standard diet (Nutrend) were similar and fell within the same range. It was observed that % protein digestibility decreased with decreasing levels of BG corresponding to decreasing protein concentration in test diet, including the standard diet. All test diets gave significantly ($p<0.05$) higher protein concentration (Pe%) when compared to the standard diet (Nutrend).

DISCUSSION

Research has shown that getting the right nutrients at the right time is critical particularly during the first 1000 days from pregnancy to the child's second birthday (GAIN and Unilever, 2013). Good nutrition leads to physical, mental and social development of an individual. This ultimately leads to the success of the individual in school, sports and in life. Inadequate nutrition leads to poor physical development and appearance and poor brain development (Nwokolo, 1987; Duke *et al*., 1977; NAS, 1979). Adequate and good nutrition of infants is very important to the development of a nation. The search for alternative but cheap and affordable infant weaning formulas is gaining popularity among African researchers and must be encouraged. Many African families live below poverty lines, and have no access to infant weaning brands in the market. They resort to unorthodox means to feed and wean their infants. These include feeding with all kinds of indigenous food materials some of which are toxic to infant body system. Simple processing techniques such as that described in this study may through advocacy and publicity aid African mothers in understanding available alternative methods for preparation of infant weaning foods at home, and may improve family lives and health. Thus, early childhood development through nutritional interventions must be encouraged in poorer regions of the world in other to achieve future populations with confidence, self belief and overall world equalities (Nwokolo, 1987; Duke *et al*., 1977; NAS, 1979).

Plant proteins are readily available in all societies. However, they are nutritionally inferior to those of animals. Studies have shown that a combination of plant proteins may provide a complete or adequate and balanced essential amino acids contents in diets (Nwokolo, 1987; Duke *et al*., 1977; NAS, 1979). The two legumes studied (AYB and BG) are relatively very cheap, readily available, affordable and easy to cultivate compared to other legumes and protein sources in Nigeria. Their nutritional benefits can be harnessed by reduction of their anti-nutrient contents through simple preparative procedures.

The role of Antinutrients in human nutrition

The anti-nutrients evaluated in this study include, phytates, tannins and cyanates. Some of their roles in human nutrition are discussed as follows.

Phytates

Phytic acid is an organic acid in which phosphorus is bound. It is commonly found in the outer hulls of nuts, seeds and grains. Phytic acid has a strong binding affinity to minerals elements such as calcium, magnesium, copper, and particularly zinc and iron. Phytates combine with these minerals in the intestinal tract and precipitates them as well as block them from being absorbed, and make them unavailable for absorption in the intestines (Ekholm *et al*., 2003, Cheryan and Rackis, 1980). Thus, a diet high in unprocessed and improperly prepared legume may lead to great mineral deficiencies and bone loss. The significant reduction of the phytate content in test diets in Table 1 shows the effectiveness of the soaking and germination techniques employed in this study. Eneobong and Obizoba (1996) observed that soaking (steeping) decreased inorganic phosphorus by 50% due to hydrolysis of bean phytate. Soaking and sprouting (Germination) reduces anti-nutrients and helps absorb the full benefits from grains. According to Abou-Arab and Abou-Salem (2009) whole seeds and grains should be soaked generally up to 12 hours to achieve significant reductions of anti-nutrients including phytates. However, Eneobong and Obizoba (1996) suggested an optimum soaking time of 12 hours, as prolonged soaking seem not to have any further effect on phytic acid reduction (Eneobong and Obizoba, 1996). The results in this study compared well with research reports about the effects of soaking and germination on the phytate contents of legumes (Chang *et al*., 1977; Ologhobo and Fetuga 1984; Akpapunam and Achinehwu, 1985; and Uzogara *et al*., 1991). In this study, phytic acid significantly ($p<0.05$) decreased during germination. Akpapunam *et al.* (1996) recorded 72 hours optimum germination time, with a percentage loss of 76% and 59% for soybean and bambara groundnut The decrease in phytic acid was attributable to increase in the phytase enzyme activity which hydrolyses the phytate complexes releasing the bound mineral phosphorus and cations which constitute the phytate structure (inositol hexaphosphate).

The phytate content of the processed test diets was lower than those of Nutrend. The processed test diets would therefore give higher nutritive value than Nutrend since they have lower phytic acid contents. Higher mineral availability would therefore be expected in the germinated seed compared to the ungerminated seeds. The appreciable loss of phytate in the processed diets suggests that the diets will be easily digested when consumed, and some essential mineral elements such as calcium, magnesium iron and zinc would be bio-available to the consumers.

Tannins

Tannins are phenolic compounds which form complexes with proteins, precipitating them and making them insoluble. Tannins inhibit and inactivate enzymes which act upon proteins causing dysfunctional digestion of proteins. Tannins reduce protein quality by decreasing digestibility. They can bind to several macromolecules like starch and reduce the nutritional value of food (Igile *et al*., 2013). Tannins also chelate metals like zinc and iron, causing decreased absorption of these elements (Igile *et al*., 2013). In general, most of these anti-nutrients help preserve the legume seeds by preventing sprouting until the right conditions are available. A variety of treatments such as milling and steaming, cooking, roasting or fermentation are used in traditional preparation of legume sources for human food (Oboh *et al*., 2006 and Seena *et al*., 2006). Preparation of legumes and grains through soaking, sprouting and germination imitates the factors that nature uses to neutralize the anti-nutrients and helps absorb the full benefit of the grains and legumes. Thus, a very high loss of tannin was observed in African yam bean (92.13%) and Bambara Groundnut (84.34%) suggesting not only that the two legumes can be used as a good protein source but also that the processing techniques of soaking and germination applied in this study effectively decreased the tannin level of the raw legumes. These results are consistent with earlier reports by Bressani *et al.* (1983); Desphande and Cheryn (1983) observed that tannins were degradable by increased activity of polyphenol oxidase during germination (Seena *et al*., 2006). Khokhar and Chauhan (1986) reported that dehulling also reduced tannin content. Reduced tannin level improved the activity of proteolytic enzymes thereby enhancing digestibility of protein and absorption of iron in the intestines (Price *et al*., 1980; Reddy *et al*., 1985).

Cyanic Acid

Cyanic acid as hydrocyanic acid bind sugar units in plants to form cyanogenic glycosides. Cyanogenic glycosides act by the decarboxylation of amino groups to give the O–glycosides. The cyano-group arises from the conjugation of α-carbon atom and amino group. Cyanide is commonly found in cereals, roots and legumes and glucosinates which prevent the uptake of iodine. These affects the proper functioning of the thyroid gland and are thus considered goitrogens.

The techniques applied in this study reduced the cyanogenic glycosides content by 60.14% and 50.04% for AYB and BG respectively. The results agreed with Aykody and Doghty (1982), who reported that germination decreased the cyanide content of lima beans by 59.50%. Also, Chinedu and Nwinyi (2012) reported that cyanogenic glycosides are reduced to tolerable levels by simple preparative procedures such as germination (a biotechnology technique). The values of the test diets were low compared to that of Nutrend (1.56mg/100g) showing higher cyanide loss compared to Nutrend. This is similar to a report by Kay (1979) who reported loss of cyanide during soaking. Loss of cyanide during germination could be attributed to the presence of degrading enzymes which act on the cyanogenic glycosides (Eka, 1980). According to Harris (2008), soaking in water neutralizes enzyme inhibitors and other substances are broken into simpler components. The levels of cyanide in processed flour, test diets and Nutrend were far below the stipulated lethal levels of 50mg/100g (Tichy, 1977) of sample by regulatory agencies around the world. Thus, the low cyanide content in test diets posed no danger of cyanide toxicity.

Oxalic Acid

Oxalic acid content found in the processed test diets was low and is below the established regulatory levels (<0.50%) in infant foods in Nigeria. Oxalate tends to render calcium unavailable by binding to plasma calcium ion to form complexes (Al-Rais *et al*., 1971). The insoluble calcium oxalate may then precipitate around soft tissues like the kidney, causing kidney stones (Oke, 1969).

In-vitro Protein Digestibility (IVPD)

The *in vitro* protein digestibility (IVPD) values of test diets and Nutrend ranged from 84.35% to 87.36% (Table 3). These values are significantly ($p<0.05$) high compared to the values obtained for raw cowpeas (76.70%) (Uzogara *et al*., 1991) and for African yam bean (62.20%) and bambara groundnut (78.0%) respectively (Edem *et al*., 1990). The significantly ($p<0.05$) high IVPD value of test diets indicated that the technique employed in this study improved digestibility of the protein samples and agrees with earlier reports of increased digestibility of processed legumes. According to Breassani *et al.* (1983) there is a negative correlation between protein digestibility and anti-nutrient factors. Germination improves digestibility due to hydrolysis of storage proteins to simple digestible units and the removal of anti-nutritional factors (Harris, 2008). The high IVPD values of test diets showed that their nutritive quality is high. The high IVPD values also suggest the diets contain good protein quality. In addition, the protein concentration (Pe%) of the test diets were significantly ($p<0.05$) higher than the recommended lower limit of 13% for plant proteins . These high Pe% values suggest that the diets are of high quality and may potentially satisfy the protein needs of people of all ages including children and adults (Kataria *et al*., 1992).

CONCLUSION

The traditional method of germination increases the nutritive quality of plant foods through reducing their anti-nutrients content. The low anti-nutrients content of test diets in this study have made them nutritionally safe and adequate as infant weaning formulas which compared favourably to reference and commercial infant food (Nutrend). The values obtained for the anti-nutritional factors are quite low and may therefore not elicit any toxicity reactions in infants. Thus, any of the test diets may be adopted in the processing of home-made infant weaning food. It was concluded that preparative processing of AYB and BG significantly reduced anti-nutrient levels in the formulation diets, improved their protein digestibility and provided alternative and cheap infant weaning food for the poor and low income households in Sub-Sahara Africa.

Suggested future Research

Further research work shall be carried out to:

1.) Determine the protein quality by investigating the amino acid profile of the processed infant food and the leguminous plant.
2.) Determine the effect of storage on the nutritive quality and shelf–life of the home-made infant food.
3.) Comparative evaluation of the nutritional potential of the processed infant food, African yam bean and bambara groundnut in laboratory animals

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