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Evaluation of Antinutrients, Minerals and Mineral Bioavailability of Water Yam Based *Ojojo* Supplemented with Bambara Groundnut

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ABSTRACT

In this study, ojojo a water yam based food was prepared, with supplementation of bamabra groundnut in the ratios of (100:0), (90:10), (80:20) and (70:30). These products were analyzed for minerals content, phytochemicals/antinutrient composition and mineral bioavailability. Results showed that, the Mineral content increased significantly (P≤ 0.05) with increase in the proportion of bambara groundnut in the product. Iron increased from 25.06 to 35.93mg/100g, magnesium from 41.15 to 43.10mg/100g, zinc from 6.80 to 7.45mg/100g, potassium from 1000 to 1100mg/100g and phosphorus from 19.50 to 58.59mg/100g. On the other hand there were significantly (P≤ 0.05) decrease in calcium from 52.12 to 48.35mg/100g and sodium from 450 to 200mg/100g. Mineral molar ratios of Na/K, Na/Mg, Ca/P and Ca/Mg decreased significantly (P≤ 0.05) while Ca/K remained constant with increase in levels of bambara groundnut supplementation. Phytochemical/antinutrient compositions of ojojo samples decreased significantly (P≤ 0.05) with increase in the level of bambara groundnut supplementation from 0-30%. Phytate decreased from 15 to 9mg/100g, saponin from 7.54 to 6.45mg/100g, flavonoid from 9.79 to 9.66mg/100g and alkaloid from 3.07 to 2.07mg/100g. On the other hand tannins increased from 3.50 to 10.00mg/100g. The phytate: mineral molar ratios for calcium, zinc, iron, and calcium/zinc decreased significantly (P≤ 0.05) with increase in the level of bambara groundnut supplementation. The study therefore suggests that supplementation of water yam based ojojo with bambara groundnut improves the mineral content, reduce antinutrient composition and improves the bioavailability of minerals.

Key words: Antinutrients, Bambara groundnut, bioavailability, minerals, ojojo, supplementation, water yam.

INTRODUCTION

Ojojo is a fried food product, produced from water yam. It is prepared traditionally by grating edible portions of water yam then adding salt and spices such as onion and pepper. It is then mixed thoroughly, scooped with spoon and fried in hot oil, which gives it a striking resemblance with *akara* balls. *Ojojo* (fried water-yam balls) is a delicacy most popular among the *Ijebu* people of south-west Nigeria. Although it was not popular amongst other tribes in Nigeria, today *ojojo* is consumed in most of the southern parts of Nigeria.

Water yam *(Dioscorea alata)*, a climbing plant with glabrous leaves and twining stems which coil readily around a stake, is a highly economical yam species Udensi *et al.*(2008). *Dioscorea alata* has been suggested to possess potential for increased consumer demand due to its low sugar content, an important factor for diabetic patients. The antinutrients in *D. alata* are low when compared to other tropical root crops Udensi *et al.* (2010). It is called *Agbo* in Tiv and *Ewura* in Yoruba land. It is the main staple food in *Ijebu* area - Western Nigeria.

Ojojo produced from water yam is low in protein, hence the need to supplement with bambara groundnut, a protein rich legume. Bambara groundnut (*Vigna subterranean*), is called Gurjiya or Kwaruru in Hausa, Okpa in Ibo and Epa-kuta in Yoruba, Igbol-ahi in Tiv parts of Nigeria. It originates from West Africa and is cultivated in drier tropical Africa. It is cultivated primarily as human food, for its seeds. In Africa, it is the third eaten legume after groundnut and cowpea (Omoikhoje, 2008). On dry basis, it consists of 51 - 70% carbohydrate, 6 - 12% moisture, 18.0 - 24.0% protein (with high lysine and methionine content), 3.0 - 5.0% ash, 5.0 - 7.0% fat, and 5.0 - 12.0% fiber. Its mineral contents are 1144 - 1935mg potassium, 2.9 - 12.0mg sodium, 95.8 - 99.0mg calcium and 4.9 - 48mg iron per 100 g seed. Especially the iron content is high compared to other legumes. Bambara groundnut can contribute positively to food security and help to alleviate nutritional problems though; it has been classified by Bamishaiye *et al.(2011)* as an underutilized crop and is only receiving more attention in the recent past.

Bioavailability is a general term that refers to how well a nutrient can be absorbed and used by the body. It can be affected by many factors such as the presence of anti-nutrients, for example, phytates, oxalates, tannins and polyphenols in foods, a person's need, fibre, competition with other nutrients and acidity of intestinal environment Paul *et al.* (2004). Minerals, classified as micronutrients are needed by our body in small amounts. Deficiency in minerals, however, can have a major impact on health such as anemia and osteoporosis that commonly occur in both developed and developing countries. There are many techniques used to determine the bioavailability of minerals in the human body. One of the methods is by measuring the molar ratio of phyate/ minerals in the food and diet (Morris & Ellis, 1989). The proportion of samples with ratios above the suggested critical values has been calculated; phytate: calcium > 0.24 (Morris & Ellis, 1985), phytate : iron > 1 Hallberg*et al.*(1989), phytate : zinc > 15 Sandberg et al.(1987), phytate : calcium/zinc > 200 Davies *et al.*(1986).

This study is aimed to estimate the mineral composition, antinutrient content and the inhibitory effect of phytate on the bioavailability of iron, zinc and calcium in water yam based *ojojo* supplemented with bambara groundnut by measuring their molar ratios.

MATERIALS AND METHODS

Raw materials

Water yam (*Dioscoreaalata*) and bambara groundnut (*Vigna subterranean*) were purchased from Wurukum market in Makurdi, Benue state. The chemicals and equipment/facilities used were obtained from food processing laboratory of Institute for Agricultural Research, Samaru, Kaduna.

Sample preparation

The water yam tubers were processed using the traditional method of processing *ojojo* which involves peeling, washing, slicing into cubes and grating with a grater of 2mm pore size. Bambara groundnut seeds were processed using modified method described by Olapade and Adetunji, 2007 which involves sorting to remove all foreign materials such as dirt, small branches and immature seeds and then cleaned by washing with water to remove dust. About 200 grams of clean seeds were soaked in cold water for 2 hours, after which it was dehulled using plate mill with 6mm clearance between the plates and then wet milled into paste. The water yam and bambara groundnut samples were mixed at the ratios of 100:0, 90:10, 80:20 and 70:30 respectively. Paste from the 100% water yam served as the control sample.

Preparation of *ojojo*

The paste for *ojojo* preparation was made by adopting standardized recipe for *ojojo* preparation by Olayiwola *et al.*(2013) with modifications. About 5g of chopped fresh pepper, 4g of chopped onions, 0.1g mono sodium glutamate seasoning, and 0.1g salt was added to 200g of the paste. The hand food blender was used to beat the mixture thoroughly. The mixture was then scooped with a spoon of volume 40ml and dispensed into a frying pot of height 8cm and radius 7.5cmcontaining 1.0 literof hot vegetable cooking oil (refined palm olein) to deep fry for 5 minutes. It was then drained and allowed to cool.

Mineral Analysis

AOAC (2005) method was used to determine the mineral composition of the samples. 1g of each sample was digested with nitric/perchloric/sulfuric acid mixture in the ratio 9:2:1, respectively and filtered. The filtrate was made up to mark in a 5ml volumetric flask. The filtered solution was loaded to an Atomic Absorption Spectrophotometer (model Buck 210; Buck Scientific, USA.). The standard curve for each mineral that is calcium, magnesium, iron, and zinc was prepared from known standards and the mineral value of samples estimated against the standard curve. Values of sodium and potassium were determined using a Flame Photometer (Sherwood Flame Photometer 410; Sherwood Scientific Ltd, Cambridge, U.K.) using NaCl and KCl as the standard while phosphorus was determined using the Vanodo-molybdate method. Mineral molar ratios were determined by dividing value of one mineral by the value of a corresponding mineral.

Antinutrient Analysis

The qualitative screening for antinutrients was carried out. The samples were screened for alkaloids, saponins, tannins, phytates, and flavonoids. Quantitative determination of phytates, tannins, saponins, alkaloids, and flavonoids were carried out in duplicates.

Determination of Alkaloid

Determination of alkaloid was made by the method described by Harborne (1973). The alkaloid content was determined gravimetrically. Five grams of the sample was weighed and dispersed in 10% acetic acid solution in ethanol to form a ratio of 1:10 (10%). The mixture was allowed to stand for 4 h at 28°C. It was later filtered via Whatman No. 42 grade of filter paper. The filtrate was concentrated to one quarter of its original volume by evaporation and treated with drop wise addition of concentrated aqueous NH_4OH until the alkaloid was precipitated. The alkaloid precipitated was received in a weighed filter paper, washed with 1% ammonia solution, and dried in the oven at 80°C. Alkaloid content was calculated and expressed as a percentage of the weight of sample analyzed.

Determination of Saponins

The spectrophotometric method was used for saponin analysis as described by Brunner (1984). One gram of the flour sample was weighed into a 250-mL beaker and 100 ml isobutyl alcohol was added. The mixture was shaken on a UDY shaker (UDY Corporation, Fort

Collins, CO) for 5 h to ensure uniform mixing. The mixture was filtered through a Whatman No. 1 filter paper into a 100-mL beaker and 20 mL of 40% saturated solution of magnesium carbonate was added. The mixture obtained was further filtered through a Whatman No. 1 filter paper to obtain a clear colorless solution. One milliliter of the colorless solution was homogenized into a 50-mL volumetric flask and 2 mL of 5% $FeCl_3$ solution was added and made up to mark with distilled water and allowed to stand for 30 min for blood red color to develop. Standard saponin solutions (0–10 ppm) were prepared from saponin stock solution and treated with 2 mL of 5% FeCl solution as done for experimental samples. The absorbance of the sample as well as standard saponin solutions were read after color development on a Spectronic 21D spectrophotometer (Milton Roy, Houston, TX) at a wavelength of 380 nm. The percentage saponin was also calculated.

Determination of total Flavonoid

This was also determined according to the method outlined by Harborne (1973). Five grams of the sample was boiled in 50 mL of 2 mol/L HCl solution for 30 min under reflux. The contents were allowed to cool and then filtered through a Whatman No. 42 filter paper. A measured volume of the extract was treated with equal volume of ethyl acetate starting with a drop. The flavonoid precipitated was recovered by filtration using weighed filter paper. The resulting weight difference gave the weight of flavonoid in the sample.

Determination of Tannin Content

Tannin content of the flour samples was determined using the methods described by Swain (1979). The sample (0.2 g) was measured in a 50-mL beaker; 20 ml of 50% methanol was added, covered with homogenizer, placed in a water bath at 77–80°C for 1 h, and the contents stirred with a glass rod to prevent lumping. The mixture was filtered using a double-layered Whatman No. 1 filter paper into a 100-mL volumetric flask using 50% methanol to rinse. This was made up to mark with distilled water and thoroughly mixed. One milliliter of the sample extract was homogenized into a 50-mL volumetric flask, and 20 mL distilled water, 2.5 mL Folin-Denis reagent, and 10 mL of 17% Na_2CO_3 were added and mixed. The mixture was made up to mark with distilled water, thoroughly mixed, and allowed to stand for 20 min when bluish-green coloration developed. Standard tannic acid solutions in the range of 0–10 ppm were treated similarly as the 1 mL sample above. The absorbance of the tannic acid standard solutions as well as samples was read after color development on a Spectronic21D spectrophotometer at a wavelength of 760nm. Percentage tannin was calculated.

Determination of Phytic Acid

An indirect colorimetric method of Wheeler and Ferrel (1971) was used for phytate determination. This method depends on an iron to phosphorus ratio of 4:6. A quantity of 5g of the test sample was extracted with 3% trichloro acetic acid. The phytate was precipitated as ferric phytate and converted to ferric hydroxide and soluble sodium phytate by adding sodium hydroxide. The precipitate was dissolved in hot 3.2 N HNO and the color read immediately at 480 nm. The standard solution was prepared from Fe $(NO_3)_3$ and the iron content was extrapolated from a Fe $(NO)_3$ standard curve. The phytate concentration was calculated from the iron results assuming a 4:6 iron: phosphorus molecular ratio.

Mineral Bioavailability

Molar ratios of anti-nutrient/minerals were used to predict the mineral bioavailability (Morris and Ellis, 1985). The molar ratios of Phytate to zinc, calcium and iron were calculated as mg/100g of Phytate intake divided by the mg/100g of zinc, calcium or iron intake respectively. The suggested critical values was given as Phytate: Calcium > 0.24 (Morris and Ellis, 1985), Phytate: Iron > 1(Hallberg et al., 1989), Phytate: Zinc > 15 (Sandberg et al., 1987) and Phytate (Calcium: Zinc) > 200 (Davies et al., 1985).

RESULTS AND DISCUSION

Mineral Composition of Water Yam Based "Ojojo" Supplemented with Bambara Groundnut

Table 1, shows the minerals composition of "ojojo" samples in mg/100gproduced from water yam-bambara groundnut blends in the ratios of 100:0, 90:10, 80:20, and 70:30. The calcium content was 52.12, 51.79, 50.06, and 48.3mg/100g respectively. The iron was 25.06, 25.70, 34.65, and 35.93mg/100g. The magnesium content was 41.15, 41.65, 42.14 and 43.10mg/100g respectively. Zinc content was 6.80, 6.87, 7.04 and 7.45mg/100g respectively. The potassium content was 1000, 1000, 1000.05 and 1100.05mg/100g respectively. The sodium content was 450.00, 400.00, 220.02 and200.01mg/100g. The phosphorus content was 19.50, 29.20, 39.85 and 58.59mg/100g. Magnesium, iron, zinc, sodium, phosphorus, calcium and potassium are essential minerals for the growth, proper formation of bones and other metabolic activities of the body, hence their selection for analysis

Zinc

Zinc plays a vital role in many biological functions such as reproduction, diabetes control, stress level, immune resistance, smell, taste, physical growth, appetite and digestion (Michael and Frank, 2004). From this research, the control (100% water yam) contained 6.80mg/100g zinc and the incorporation of bambara groundnut of 10%, 20% and 30% significantly affected its zinc content by increasing it to 6.87, 7.04, and 7.45mg/100grespectively, due to appreciable amount of zinc (8.10mg/100g) in bambara groundnut Abdulsalami *et al.* (2010) and the low zinc content (0.8mg/100g) and (1.01-1.76mg/100g) in water yam as reported by Lowell *et al.* (2012) and Faustina *et al.* (2013) respectively.

Iron

Iron (Fe) is an essential component of such protein as hemoglobin, myoglobin and ferritin; and some enzymes require Fe as a co-factor (Fairbanks, 1999). Iron in heme allows the transport of oxygen to tissues (hemoglobin), transitional storage of oxygen in tissues (myoglobin), and the transport of electrons through the respiratory chain (cytochromes). The daily iron requirement for men, non-menstruating and pregnant women is 18mg/100g (FAO, 2000). The iron content of 100% *ojojo* was seen to be high (25.06mg/100g). The supplementation of bambara groundnut up to 30% increased its iron content to 35.93mg/100g, significantly increasing the iron content of the *ojojo*. This result shows that water yam has lower iron content (1.5mg/100g) as reported by Lowell *et al.* (2012) than bambara groundnut with 18.51mg/100g as reported by Oyeleke *et al.* (2012) and 25.10mg/100g as reported by Abdulsalami *et al.* (2010), hence it is a better supplier of iron and it is in agreement with earlier reports by Mkadawire (2007) who reported that bambara groundnut contains 4.9-48mg/100g of iron.

Magnesium

Magnesium helps to regulate muscles and nerve function and influences the metabolism of proteins, carbohydrates, fats and nucleic acids (Shils, 1999). The magnesium content of the control (100% water yam) was determined to be 41.15mg/100g. Supplementation with bambara groundnut increased the magnesium content of *ojojo* significantly ($p \leq 0.05$) to 43.10mg/100g at 30% bambara groundnut supplementation. This was due to the relatively high magnesium content of bambara groundnut 347.15mg/100g as reported by Oyeleke *et al.* (2012) than in water yam (60.77-97.27mg/100g) as reported by Udensi *et al.* (2008).

Potassium

Potassium helps to maintain osmotic pressure and acid-base balance. The potassium content of the control (100% water yam) was determined to be 1000.00mg/100g. This was significantly ($p \leq 0.05$) increased to 1100.05mg/100g when bambara groundnut was incorporated up to 30%. This could be because water yam has lower potassium content (240-400mg/100g) as reported by Udensi *et al.* (2008) while bambara groundnut has 1702.10mg/100g as reported by Oyeleke *et al.* (2012).

Phosphorus

Phosphorus is widely distributed in soft tissues and is required to drive multiple metabolic and energy reactions within and between cells. As phosphate, it helps to maintain osmotic and acid-base balance. As a phospholipid, it contributes to cell membrane fluidity and integrity (Knochel, 1999). This work shows that 19.50mg/100g phosphorous is contained in the 100% water yam *ojojo*. This concentration of phosphorous was significantly ($p \leq 0.05$) increased to 58.59mg/100g as supplementation with bambara groundnut increased to 30%. This is an indication that bambara groundnut (738.04mg/100g) as reported by Oyeleke *et al.* (2012) is a better supplier of phosphorus than water yam (100-430mg/100g) as reported by Udensi*et al.* (2008).

Calcium

Calcium is needed for the development of teeth and bones and it binds to many cellular proteins, resulting in their activation. The calcium content of the control (100% water yam) was determined to be 52.12mg/100g, this was significantly ($p \leq 0.05$) reduced to 48.35mg/100g when bambara groundnut was incorporated from 10% up to 30%. This could be because water yam has higher calcium content (20.40-80.16mg/100g) as reported by Udensi *et al.* (2008) than bambara groundnut with calcium content of 18.51mg/100g as reported by Oyeleke *et al.* (2012). Similar result was reported by Olayiwola *et al.* (2012) for cocoyam based *ojojo* supplemented with cowpea.

Sodium

Sodium helps to regulate thirst and total body water. Sample prepared from 100% water yam contained 450.00mg/100g sodium and the incorporation of bambara groundnut of 10%, 20% and 30% levels of supplementation significantly affected its sodium content by decreasing it to 400.00, 220.02 and 200.01mg/100g respectively, in line with the low sodium content of bambara groundnut. This is in agreement with the work of Oyeleke *et al.* (2012) and Udensi *et al.* (2008) who separately reported the value of 135.30mg/100g sodium in bambara groundnut and 190-380mg/100g sodium in water yam respectively.

From this research, the values of iron, magnesium, zinc, potassium and phosphorus increased with increased in the proportion of bambara groundnut while the values of calcium and sodium decreased. This is an indication that bambara groundnut could be a better provider of iron, magnesium, zinc, potassium and phosphorus than water yam although, only the levels of iron met the 18mg/100g RDA for infants, children and adults.

Mineral Molar Ratio of Water Yam Based "Ojojo" Supplemented with Bambara Groundnut

Table 2 shows the result of mineral molar ratio of "ojojo" samples produced from water yam-bambara groundnut blends in the ratios of 100:0, 90:10, 80:20, and 70:30. The sodium: potassium molar ratio was 0.45, 0.40, 0.21 and 0.18 respectively. The calcium: phosphorus molar ratio was 2.67, 1.74, 1.26 and 0.83. The calcium: potassium molar ratio was 0.05 in all the samples. Sodium: magnesium molar ratio was 10.94, 9.60, 5.22, and 4.64 respectively. The calcium: magnesium molar ratio was 1.27, 1.24, 1.19 and 1.12 respectively. The values of Ca/K remained constant while Na/K, Na/Mg, Ca/P and Ca/Mg decreased with increase in the level of bambara groundnut supplementation in the "ojojo" samples.

Calcium: Phosphorus Molar Ratio (Ca/P)

The Calcium/Phosphorus molar ratio was 2.67 for the control sample and decreased to 0.83 for sample with 30% bambara groundnut supplementation. The Ca/P ratio was generally up to the 1.00 except the sample containing 30% bambara groundnut. It is well known that diets with high value of Ca/P ratio are considered "good," if the Ca/P > 1 and "poor" if Ca/P < 0.05 particularly for growing children who require high intake of calcium and phosphorus for bone and teeth formation Nieman *et al.*(1992). Although supplementation with bambara groundnut showed decrease in Ca/P molar ratio, only the value of 30% bambara groundnut supplementation was below the >1 recommended limit. However, all the samples were in the recommended "good" zone.

This study indicates that water yam based *ojojo* supplemented with bambara groundnut is of nutritional benefit, particularly for children and the aged who need higher intakes of calcium and phosphorus for bone formation and maintenance

Sodium: Potassium Molar Ratio (Na/K)

Sodium/Pottatium molar ratio range from 0.45 for the control sample (100% water yam) to 0.18 for sample with 30% bambara groundnut supplementation. The Na:K ratio < 1 is recommended for diets, particularly for hypertensive patients Nieman *et al.* (1992). Therefore, the observed Na/K molar ratio of *ojojo* samples in this study shows that *ojojo* is suitable for people who have the risk of high blood pressure, hypertension. This is in conformation with earlier work of Osagie and Eka, (1998) who suggested that water yam is suitable for people with high blood pressure.

Calcium: Potassium Molar Ratio (Ca/K)

Calcium/Potassium of 4.2:1 or less is recommended Nieman *et al.* (1992), however, the values for this research in all the samples were far below (0.05 for all the samples) and hence low thyroid functioning may not occur from consuming *ojojo.*

Sodium: Magnesium Molar Ratio (Na/Mg)

From this study, values of sodium/potassium were higher than the Na/Mg < 4 recommended by Nieman *et al.* (1992) and could be attributed to the low levels of magnesium in the samples of *ojojo*. The values of Na/Mg steadily decreased with increase in bambara groundnut supplementation from 10.94 to 4.64 at 30% level of supplementation. This could be because of the decrease in sodium and increase in magnesium with increase in the quantity of bambara groundnut. However, it was observed that the decrease was beneficial as the result tends towards the required Na/Mg ratio of less than 4.

Calcium: Magnesium Molar Ratio (Ca/Mg)

The values of Ca/Mg decreased with increase in the proportion of bambara groundnut and the values ranged from 1.12 to 1.27. Hence from the result of this research, it is shown that *ojojo* samples were below the range of 3:1(simple carbohydrate sensitivity range). This could lead to calcium precipitating in tissues as reported by Niema *et al.* (1992).

Anti-Nutrient Composition of Water Yam Based "Ojojo" Supplemented with Bambara Groundnut

Table 3 shows the anti-nutrient composition of "ojojo" of samples prepared from water yam-bambara groundnut blends in the ratios of 100:0, 90:10, 80:20 and 70:30. The tannins content ranged from 3.5mg/100g to 10.0mg/100g. The phytate content ranged from 9mg/100g to 15mg/100g. The alkaloid content ranged from 2.07mg/100g to 3.07mg/100g. The flavonoid content ranged from 9.66 to 9.79mg/100g. The saponins contents ranged from 6.44 to 7.53mg/100g. The values of tannins increased while phytate, flavonoid, alkaloid and saponins decreased with increase in the quantity of bambara groundnut supplementation.

Tannins, Saponins and Alkaloid are known to have anti-microbial activities as well as other physiological activities (Sofowora, 1980; Evans, 2005). However, from this research, the value of the phytate, alkaloid, flavonoid and saponnins decreased while tannins increased with a corresponding increase in the quantity of bambara groundnut.

Tannins

Sample prepared from 100% water yam had the least tannins content of 3.5mg/100g while sample with 30% bambara groundnut supplementation had the highest mean value 10.00mg/100g. However, there was no significant difference between all the samples at $P \leq 0.05$ level of significance. Increase in tannin could be as a result of bambara groundnut which has high tannin content of 7.15mg/100g as reported by Oyeleke *et al.* (2012).

Phytates

The level of phytate decreased with increase in supplementation as the values of phytate for the control had the highest value of 15mg/100g while sample with 30% bambara groundnut supplementation having the least mean value of 9mg/100g. This reduction could be as a result of bambara groundnut having a relatively low phytate (6.94mg/100g) as reported by Oyeleke *et al.* (2012). However, the values from this research are lower than the 25mg/ 100g recommended by Onomi *et al.* (2004) for minimal micronutrient loss with no adverse effects on digestibility as reported by Nwakolo and Bragg, (1997) and therefore does not have adverse effect on digestibility. The control was however significantly different from all other samples.

Saponin

Sample prepared from 100% water yam had the highest means value of 7.54mg/100g while sample with 30% bambara groundnut supplementation had the least mean value of 6.45mg/100g. There was however, no significant different between the control and sample with 10% bambara groundnut supplementation at $P \leq 0.05$ level of significance. Their presence show that *ojojo* could be of health benefit Harborne, 1984; Okwu, (2001) reported that saponins have anti-hypercholesterol, anti-inflammatory, cardiac depressant properties and also appears to kill or inhibit cancer cells without killing any cells in the process. Their values are within tolerant levels and may not impair the availability of glucose and cholesterol as reported by (Okaraonye and Ikewuchi, 2009).

Flavonoid

From this research, the control had the highest mean value of 9.86mg/100g while sample with 30% bambara groundnut supplementation had the least mean value of 9.66mg/100g. However, there was no significant difference between all the samples at $P \leq 0.05$ level of significance. The flavonoids have long been recognized to possess anti-inflammatory, antioxidant, antiallergic, antithrombotic, antiviral, and anti-carcinogenic activities Carroll *et al.* (1998). There has been no dietary reference intake (DRIs) from the National Academy of Sciences and there is no daily value (DV) from the U.S. Food and Drug Administration as there has been no evidence of direct toxicity caused by dietary flavonoid (Chun *et al.*, 2007).

Alkaloids

Alkaloid decreased with increase in bambara groundnut substitution. Sample prepared from 100% water yam had the highest mean value of 3.0mg/100g while sample with 30% bambara groundnut supplementation had the least mean value of 2.07mg/100g. However, samples with 10 and 20% bambara groundnut supplementation were not significantly different at $P \leq 0.05$ level of significance. From this research, the values of alkaloid were lower than the upper limit of 60mg/100g recommended for safe feed McDonald *et al.* (1995). Hence the alkaloid content does not possess any threat to the consumer.

The level of anti-nutrients in the *ojojo* samples in this work is considered to be non-toxic. Thus, the result reveals that the anti-nutrient composition of *ojojo* samples were generally low hence that none of the anti-nutrient values was above the lethal dosage approved by standard bodies like National Agency for Food and Drug Administration and Control (NAFDAC) in Nigeria as reported by Blessing *et al.* (2011).

Also the amount of phytate and tannins reported in this study were within the range that would not adversely affect nutritional values or cause any toxic effects associated with anti-nutrients. This could imply that consuming water yam *ojojo* supplemented with bambara groundnut will not affect human nutrition adversely when large quantity is consumed and is therefore an advantage to the consumer of *ojojo*.

Anti-Nutrient/Mineral Molar Ratio of Water Yam Based "Ojojo" Supplemented with Bambara Groundnut

Table 4 shows the ant-inutrient: mineral molar ratio of "ojojo" samples prepared from water yam-bambara groundnut blends in the ratios of 100:0, 90:10, 80:20 and 70:30. The phytate: calcium molar ratio ranged from 0.18 to 0.28. The phytate: iron molar ratio ranged from 0.25 to 0.60. The phytate: zinc molar ratio ranged from 1.28 to 2.21. The phytate (calcium: zinc) molar ratio ranged from 58.41 to 114.99. The values of anti-nutrient: mineral molar ratio decreased with increase in the quantity of bambara groundnut supplementation in all the treatments. Zinc, iron and calcium are essential minerals that are often lacking in human diets, either due to sufficient intake or due to poor absorption (Andinet and Getachew, 2016). The influence of phytate on the bioavailability of minerals depends not only on the phytate content of the diet but also on the interactions between phytate and minerals.

Molar Ratio of Phytate to Zinc

The calculated phytate/zinc molar ratios for *ojojo* samples were within the range of 1.21 to 2.21. All the samples of *ojojo* were in the range of the suggested critical level (<15 regarded as favorable for zinc absorption) Morris and Ellis, 1985. According to WHO cut-offs for phytic acid to zinc molar ratios >15, 5-15 and 5 is equal to zinc bioavailability as low, moderate and high respectively (Goicoechea, 1996). In this context, all the *ojojo* samples had high zinc bioavailability. Hence it was observed that supplementation of *ojojo* with bambara groundnut will improve the bioavailability of zinc as the values keeps decreasing with increase in supplementation with bambara groundnut.

Molar ratio of Phytate to Iron

Phytate/iron molar ratios were < 1 (indicative of good iron bioavailability) as reported by Hallberg *et al.*(1989) for all *ojojo* samples as the ratios range from 0.25 in 30% bambara groundnut supplemented *ojojo* to 0.60% in the control sample. Although from this research, results showed that the values of phytate: iron was within the recommended level, however, it was evident that supplementation of *ojojo* with bambara groundnut improved the level of bioavailability of iron as the values keeps decreasing.

Molar Ratio of Phytate to Calcium

Results showed that only the 100% water yam *ojojo* sample was greater than the recommended 0.24 which will not impair calcium availability as reported by (Morris and Ellis, 1985). The values of phytate/calcium, however ranged from 0.19 in 30% bambara groundnut supplemented *ojojo* to 0.29 in the control and it could be seen that supplementation with bambara improved the bioavailability of calcium as the values keeps decreasing.

Molar Ratio of Phytate to Calcium/Zinc

The ratios for all the samples were < 200 as they ranged from 58.41 in 30% bambara groundnut supplemented *ojojo* to 114.97 in the control (100% water yam) thus they have good phytate /(calcium: zinc) molar ratios as it was suggested by Davies *et al.* (1985). However from the results it was shown that supplementation of *ojojo* with bambara groundnut improved bioavailability as the values keeps decreasing.

From this research, the "good" bioavailability of zinc, iron and calcium could be due to the low content of phytate in the food (9.00-15.00mg/100g) which did not affect the mineral bioavailability as reported by Nwanna *et al.* (2005). There was however improvement in the bioavailability of minerals with supplementation with bambara groundnut and this could be because of the decrease in phytate levels (15mg/100g to 9mg/100g) with increase in the proportion of bambara groundnut in the *ojojo* samples.

CONCLUSION

This work has shown that there is improvement in the mineral content of "*ojojo*", through the use of bambara groundnut supplementation, as it offers improvements in minerals and mineral molar ratios especially iron, magnesium, zinc, potassium, phosphorus, Ca/P, Na/K and Ca/Mg than when only water yam was used. The work shows a decrease in the anti-nutrient content as evident in the decrease of phytate, alkaloid, flavonoid and saponins content and increase in the bioavailability of zinc, iron and calcium.

Table 1: Mineral Composition of Water Yam-Based 'Ojojo' Supplemented with Bambara Groundnut (mg/100 g)

Parameter LSD		Ojojo			
	WY	100	90	80	70
	BGN	0	10	20	30
Calcium	1.71	52.12^{a}	51.79^{a}	50.06^{b}	48.35^{c}
Iron	0.64	25.06^{d}	25.70^{c}	34.65^{b}	35.93^{a}
Magnesium	0.49	41.15^{d}	41.65^{c}	42.14^{b}	43.10^{a}
Zinc	0.65	6.80^{d}	6.87^{c}	7.04^{b}	7.45^{a}
Potassium	50.00	1000.00^{c}	1000.00^{c}	1050.05^{b}	1100.05^{a}
Sodium	20.01	450.00^{a}	400.00^{b}	220.2^{c}	200.01^{d}
Phosphorus	9.70	19.50^{d}	29.20^{c}	39.85^{b}	58.59^{a}

Values are means of duplicate determinations. Mean values along the same rows with different superscripts are significantly different ($P \leq 0.05$).
WY= Water yam, BGN= Bambara groundnut, LSD= least significant difference.

Table 2: Mineral Molar Ratio of Wateryam-based 'Ojojo' Supplemented with Bambara Groundnut

Parameters	LSD	Ojojo			
	WY	100	90	80	70
	BGN	0	10	20	30
Na/K	0.03	0.45^{a}	0.40^{b}	0.21^{c}	0.18^{d}
Ca/P	0.43	2.67^{a}	1.74^{b}	1.26^{c}	0.83^{d}
Ca/K	0.00	0.05^{a}	0.05^{a}	0.05^{a}	0.05^{a}
Na/Mg	4.64	0.42	10.94^{a}	9.60^{b}	5.22^{c}
Ca/Mg	0.03	1.27^{a}	1.24^{a}	1.19^{b}	1.12^{c}

Values are means of duplicate determinations. Mean values along the same rows with different superscripts are significantly different ($P \leq 0.05$).Na= Sodium, K= Potassium, Ca= Calcium, P = Phosphorus, Mg = Magnesium, WY= Water yam, BGN= Bambara groundnut,LSD= least significant difference.

Table 3: Anti-nutrient Composition of Wateryam-based "Ojojo"Supplemented with Bambara Groundnut (mg/100 g).

Parameter	LSD	Ojojo			
	WY	100	90	80	70
	BGN	30	0	10	20
Tannins	0.25	3.52^{a}	5.74^{a}	7.59^{a}	10.00^{a}
Phytate	0.24	15.03^{a}	13.00^{ab}	10.50	9.07^{b}
Alkaloid	1.00	3.07^{a}	2.68^{ab}	2.32^{ab}	2.07^{b}
Flavonoid	0.02	9.79^{a}	9.74^{a}	96.73^{a}	9.66^{a}
Saponins	0.40	7.53^{a}	7.53^{a}	7.08^{b}	6.44^{c}

Values are means of duplicate determinations. Mean values along the same rows with different superscripts are significantly different ($P \leq 0.05$).

WY= Water yam, BGN= Bambara groundnut, LSD= least significant difference.

Table 4: Anti-nutrient: Mineral Molar Ratio of Water yam-based 'Ojojo' Supplemented with Bambara Groundnut

Parameter	LSD	Ojojo			
	WY	100	90	80	70
	BGN	0	10	20	30
Phytate: Calcium	0.01	0.28^{a}	0.25^{b}	0.19^{c}	0.18^{d}
Phytate: Iron	0.03	0.60^{a}	0.51^{b}	0.29^{c}	0.25^{d}
Phytate: Zinc	0.22	2.21^{a}	1.89^{b}	1.42^{c}	1.21^{c}
Phytate: ca/zn (mg/100g)	114.99^{a}	98.01^{b}	71.11^{c}	58.41^{d}	18.4

Values are means of duplicate determinations. Mean values along the same rows with different superscripts are significantly different (P≤0.05). Ca = Calcium, Zn =Zinc, WY= Water yam, BGN= Bambara groundnut, LSD= least significant difference.

REFERENCES

Abdulsalami, M. S., Sheriff, H. B. (2010). Effect of Processing on the Proximate Composition and Mineral Content of Bambara Groundnut (Voandzeiasubterrenea). *Bayero Journal of Pure and Applied Science*, 3:188-190.

Andinet, A. H. and Getachew, A. (2016). The Content and Bioavailability of Mineral Nutrients of Selected Wild and Traditional Plants as Affected by Household Preparation Methods Practiced by Local Community in BenishangulGumuz Regional State, Ethiopian Public Health Institute, Adis Ababa, Ethiopia. *International Journal of Food Science*. Vol 2, pp 174-174.

AOAC. (2005). Official Methods of Analysis. Association of Official Analytical Chemists, 18th Edn.Washington D.C-USA.

Bamishaiye, O. M., Ajebola, J. A and Bamishaiye, E. I. (2011). Bambara Groundnut; an Underutilized Nut in Africa. Advances in Agricultural Biotechnology. 1:60-72.

Blessing, A. C., Ifeanyi, U. M. and Chijioke, O. B. (2011). Nutritional Evaluation of Some Nigeria Pumpkins (*Cucurbitaspp*).Fruit, Vegetable and Cereal Science and Biotechnology. Global Science Books.5(2):64-71

Brunner, J. H. (1984). Direct Spectrophotometric Determination of Saponins. *Analytical Chemistry*. 34:1314-1326.

Carroll, D and Seers, k. (1998). Relaxation for the relief of chronic pain: a systematic review. Journal of Advanced Nursing. pp 476-487.

Chun, O.K., Chung, S.J. and Song, W.O. (2007).Estimated dietary flavonoid intake and major food sources of U.S. *Adults Journal of Nutrition*. 137:1244-52.

Davies, N.T. (1982). Effects of Phytic Acid in Mineral Availability, In: Vahomg, G.V, Kritchersky, K (eds). Dietary Fiber in Health and Disease. Plenum Press. NY, USA, pp 99, 105-116.

Evans, W.S (2005). Trease and Evans Pharmacognosy. 15th ed. Elsevier, India. pp 1869-1878.

Fairbanks, V.F. 1999. Iron in medicine and nutrition; In Modern Nutrition in Health and Disease, 9th edition. pp 193-221.

FAO.(2000). Production Yearbook, Volume 55, Collection FAO Statistics, no. 170.FAO, Rome, Italy.

Faustina, D. W. M., Ibok, O., William, O. E., Robert, A and Bussie, M. D. (2013). Potential Health Benefit of Water yam (Discoreaalata). *Food Function*. 4: 1496-1501.

Goicoechea, J. (1996). World Health Organization and Regional Office for Europe, Primary Health Care Reforms, World Health Organization, Geneva, Switzerland. 315, pp173.

Hallberg, L, Rossander, L and Skanberg, A.B. (1989). Phytate and the inhibitory effect of bran on iron absorption in man. *American Journal of Clinical Nutrition*. 45: 988-996.

Harborne, J. B. (1973). *Phytochemical Methods; A Guide to Modern Techniques Plant Analysis*. Chapman and Hall Ltd, London, Uk. pp278.

Harborne, J. B. (1984). *Phytochemical Methods*. London Chapman and Hall Ltd pp 49-188.

Knochel, J. P. (1999). *Phosphorus; In Modern Nutriton in Health and Disease*, 9thed pp 157-167.

Lowell, D., Kesi, B., Racquel, W., Mitchelle, O., Sannette, H. and Helen, A. (2012). Antioxidants, Minerals and Bioactive Compounds in Tropical Staples. *African Journal of Food Science and Technology*. 3(4): 90-98.

McDonald, P., Edward, R. A., Greenhalgh, J. F. D and Morgan, C. A. (1995). Animal Nutrition 5th ed. Longman Singapore Publisher (Pte) Ltd Singapore, 74(5): 70-92.

Michael, H.W and Frank, J.F. (2004). Standard Methods for the Examination of Dairy Products. 17thed, American Public Health Association, Washington, USA. pp547.

MkandawireCeasar. (2007). Review of Bambara Groundnut (Vigna subterranean (L.)Verdc. Production in Sub-sahara Africa. *Agricultural journal* 2 (4): 465-470

Morris, E. R. and Ellis, R. (1985). Bioavailability of Dietary Calcium-Effect of Phytate on Adult Men Consuming Non-vegetarian Diets. In Kies, C. (ed): ACS Symposium Series 275: Nutritional Bioavailability of Calcium. *American Chemical Society*: Washington, DC, USA, pp 63.

Nieman, D. C., Butterworth, D. E., Niemen, C. N. (1992). *Nutrition*. WM.C. Brown, Dubuque.

Nwanna, L. C., Enujiugha, V. N., Oseni, A. O. and Nwanna, E. E. (2005). Possible Effects of Fungal Fermentation on Bambara Groundnuts (Vigna subterranean (L.) Verde) as feedstuff resources. *Journal of Food Technology*. 3(4): 572-575.

Nwokolo, E. N. and Bragg, B. B. (1977). Influence of Phytic Acid and Crude Fiber on the Availability of Minerals from Protein Supplements in Growing Chicks. *Journal of Animal Science*. 57:475 – 477.

Okaraonye, C. C. and Ikenachi, J. C. (2009).Nutritional and Anti-nutritional Components of *Pennisetum purpurium* (schumech). *Pakistan Journal of Nutrition* 8(11): 32-34.

Okwu, D. E. (2001).Evaluation of chemical composition spices and flavouringagents. *Global Journal pure and Applied Science*. 7:455-459.

Olapade, A. and Adetunji, D. O. (2007). Comparison of Different Methods of Producing Bambara (Voandzeia subterranean L. Thou) Flours for Preparation of 'moinmoin' *Nigerian Food Journal*. 25(2).

Olayiwola, I., Folaranmi, F., Abdul-Rasaqi, A., Oluseye, O., Ajoke, A and Wasiu, A. (2013). Chemical, Mineral Composition and Sensory Acceptability of Cocoyam-based Recipes Enriched with Cowpea Flour. *African Journal of Food and Nutrition,* 13: 228-234.

Omoikhoje, S. O. (2008). Assessment of the Nutritive Value of Bambara Groundnut as Influenced by Cooking Time. *Livestock* reseairch *for Rural Development*. 20(4)

Onomi, S., Okazaki,Y. and Katayama, T. (2004). Effect of Dietary Level of Phytic Acid on Heptic and Serum Lipid Status on Rat Fed a High Sucrose Diet. *Bioscience Biotechnology Biochemistry* 68:1379-1381.

Osagie, A. U. and Eka, O. U. (1998).Chemical Composition of Root and Tuber crops. In: Nutritional Quality of Plant Foods. Department of Biochemistry, University of Benin, Benin City Nigeria.

Osagie, A.U. (1992). The Yam Tuber in Storage. Postharvest Research Unit, University of Benin, Benin City, pp: 33-84.

Oyeleke, G.O., Afolabi, O and Isola, A. D. (2012). Some Quality Characteristics and Carbohydrate Fractions of Bambara Groundnut Seed Flour. *IOSR Journal of Applied Chemistry.* 2(4):16-18.

Sandberg, A. S., Anderson, H., Carlesson, N. G., and Sandstorm, B. (1987). Degradation Products of Bran Phytate Formed during Digestion in the Human Small Intestine: Effects of Extrusion Cooking on Digestibility. *Journal Nutrition*. 117, 2061-2065.

Shils, M. E. 1999. *Magnesium; In Modern Nutriton in Health and Disease*, 9thed pp, 169-192.

Sofowora, A. (1980). Guidelines for Research Promotion and Development in Traditional Medicine. *Nigerian Journal of Pharmacy*. 11:117-118.

Swan, T. (1979). Tannins and Ligins; In Rosenthal. G and Janven, D.H. eds. Herbivores: their Interactions with Plant Metabolites. *Academic Press*, New York, NY. Pp 657-682.

Udensi, E. A.,Oselebe, H. O. and Iweala, O. O.(2008). The Investigation of Chemical Composition and Functional Properties of Water yam (Dioscoreaalata): Effect of Varietal Differences. *Pakistan Journal of Nutrition* 7: 342-344.

Udensi, E. A.,Oselebe, H. O. and Onuoha, A. U. (2010). Anti-nutritional Assessment of D. alata varieties. *Pakistan Journal of Nutrition* 9: 179-181.

Wheeler, E. I. and Ferrel, R. E. (1971). A method for Phytic Acid Determination in Wheat and Wheat Fractions. *Cereal Chemistry*. 48: 312-320.