Effect of soaking time on the nutrient and antinutrient composition of bambara groundnut seeds (*Vigna Subterranean*)

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ABSTRACT

The study was designed to investigate the effect of different soaking time on the nutrient and antinutrient composition of bambara ground nut (*Vigna subterranean*) seeds. Milky coloured variety of bambara groundnut was purchased from Ubani Market Umuahia, Abia State. The sample was cleaned by winnowing. One kilogram of whole seeds was soaked in potable water at room temperature (29-32^{o}C in a 1: 30 (bean: water) ratio for 6, 12, 18, 24, 48 and 72 hrs respectively. The soaked seeds were drained, rinsed, dehulled and dried at 55^{0}C in an oven until the moisture became constant. Milling the dried seed into flour was done using attrition milling machine. The proximate, vitamin and anti-nutrient content of the samples were determined using standard methods. Mineral elements were determined using wet-acid digestion method for multiple nutrients determination. All tests were carried out in duplicates and the data generated were analysed using standard methods. The moisture content of the samples ranged between 10.2 -11.2%. Values of protein (20.2-22.4%), crude fiber (4.1- 4.8%), crude fat (5.9-7.2%), and ash (3.6-3.9%) decreased with increasing soaking time. The result shows decrease of 2.85%- 12.4% for Ca, 1.54%-36.2% for Mg, 4.27%-16.2% for Na, 2.24%-11.2% for K, 6.21%-22.0% for Fe, 23.8%-90.0% for Cu, 14.28%- 64.28% for Se and 12.9%-44.5% for I respectively. There was significant decrease in all the vitamins except for vitamin B_2 in the sample soaked for 24hr and in the sample soaked for 48hr (0.09mg/100g, 0.08mg/100g respectively). It was found that all the anti-nutrient composition of un-soaked and soaked bambara groundnut were within permissible limit which implies that soaking bambara

Keywords: Soaking time, Bambara groundnut, Nutrient, Antinutrient

INTRODUCTION

Legumes are known as important cheap sources of protein to poor resource people in Nigeria (Adebayo, 2014); they are next important crops after cereals (Uzoechma, 2009). Their seeds have an average of twice as much protein as cereals (Vijaykumarri *et al*., 1997; Udensi *et al*., 2010) The major drawback however in the use of most legume is the fact that the seeds are not easily rehydrated; they are difficult to cook; the seed coat is difficult to remove (Urga *et al*., 2006). Long soaking time is required by traditional processing methods to remove seed coat of the grain.

Bambara groundnut is a legume crop native to Africa commonly grown for its seeds by subsistence farmers. It is grown in many parts of Africa, Asia, Indonesia, and South America (Linnemann and Azam-Ali, 1993; National Research Council, 2006; Basu *et al*., 2007). Bambara groundnut (*Vigna subterranean*) belongs to the family of fabaceae. It is an annual herbaceous, intermediate plant with creeping stems. The nuts are known as jugo beans (South Africa), ntoyo ciBemba (Republic of Zambia), Gurjiya or Kwaruru (Hausa, Nigeria), Okpa (Ibo, Nigeria), Epa-Roro (Yoruba, Nigeria) and Nyimo beans (Zimbabwe) (Bamishaiye e*t al*., 2011).The colour of the seeds vary from white, cream, red, black and in some cases mottled with colours such as brown, red or black (Amarteifio *et al*., 2010). The crop is known for its

tolerance to drought, relative resistance to pests, diseases and the ability to produce yield in poor soils too poor to support the growth of other legumes (Brough and Azam-Ali, 1992). The seed ripe or immature Bambara groundnut (*Vigna subterranean*) contains about 20% protein, 60% carbohydrates and 7% oil (Goli, 1995; National Research Council, 2006). Lysine and Leucine are the predominant essential amino acids found in *Vigna subterranean* (Mune *et al*., 2011; Mazahib *et al*., 2013). While the predominant fatty acid found in Bambara groundnut are linoleic, palmitic and linolenic acids (Minks and Bruneteau, 2000). In Nigeria mature fresh bambara groundnut is boiled and consumed as snack while mature dried ones are either toasted and consumed as snack or prepare into ukpa; the matured dried seeds are however, now used in Nigeria to produce milk (Udeze *et al*., 2014) and like other legume, bambamra groundnut is also soaked in water to enhance cleaning and to bring to a safe level its anti-nutritional level before been processed into milk. There is however limited information on soaking effects on the nutrient and anti-nutrient contents of bambamra groundnut seeds. This study was designed to investigate the effect of soaking time on the nutrients and antinutrients composition of bambara groundnut seeds.

MATERIALS AND METHODS

Source of materials/ Preparation

Milky coloured variety of bambara groundnut (*Vigna subterranean*) was purchased from five randomly selected vendors from the Ubani Market Umuahia, Abia State and pooled to obtain the sample for analysis. The sample was cleaned by winnowing, and removing of extraneous objects and immature seeds. One kilogram of whole seeds was soaked in potable water at room temperature (29-32^{o}C in a 1: 30 (bean: water) ratio for 6, 12, 18, 24, 48 and 72 hrs respectively. The soaked seeds were drained, rinsed, dehulled and dried at 55^{0}C in an oven until the moisture became constant. Milling the dried seed into flour was done using attrition milling machine (Thomas Wiley Model ED-5) to 5mm sieve size. The milled samples were stored in air-tight container for further analysis.

Chemical analyses

The proximate compositions of the sample were determined using standard A.O.A.C. (2006) methods. Moisture content of the jam was determined gravimetrically. The crude protein content was determined by micro-Kjeldahl method, using 6.25 as the nitrogen conversion factor. The crude fat content was determined by Soxhlet extraction method using petroleum ether. The ash content was determined by incinerating the samples at 600^{o}C in a muffle furnace. Carbohydrate was obtained by difference, while energy was calculated using the Atwater Conversion factors in KJ and Kcal (17KJ/4Kcal, 17KJ/4Kcal, and 37KJ/9Kcal, for protein, carbohydrate and lipid respectively).

Mineral elements were determined using wet-acid digestion method for multiple nutrients determination as described by the method of A.O.A.C (2006). About 0.2g of the processed sample material was weighed into a 150ml Pyrex conical flask. Five (5.0) ml of the extracting mixture (H_2SO_4 – Sodium Salicylic acid) was added to the sample. The mixture was allowed to stand for 16 hours. The mixture was then placed on a hot plate set at 30^{o}C and allowed to heat for about 2hours. Five (5.0) ml of concentrated perchloric acid was introduced to the sample and heated vigorously until the sample was digested to a clear solution. Twenty (20) milliliters of distilled H_2O was added and heated to mix thoroughly for about a minute. The digest was allowed to cool and was transferred into a 50ml volumetric flask and made up to the mark with distilled water. The digest was used for the determinations of calcium (Ca) and magnesium (Mg) by the ethylamine ditetra acetic acid (EDTA) versanate complexiometric titration method. Potassium (K) and sodium (Na) were evaluated by flame photometry method and phosphorus (P) by the vanadomolybdate method using the spectrophotometer. The trace metals (zinc, iron, copper, selenium, manganese and iodine) were determined using the atomic absorption spectrophotometer 969 instrument. The appropriate cathode lamp was fixed for each element. The sample was introduced to the atomizer and the value concentration of the element printed out as mgX/liter.

The β – carotene, riboflavin, niacin and thiamin of the products were determined spectrophotometrically as described by AOAC (2006). Ascorbic acid was determined as described by AOAC (2006) using titration method. Gravimetric method as described A.O.A.C (2006) was used to determine alkaloids. Saponin and was determined by gravimetric oven drying method as described by the method of Obadoni and Ochuko (2001). Tannin content of the sample was determined spectrophometrically as described (Krik and Sawyer, 1998). Phenol was determined by the folin-ciocatean spectrophotometry method (AOAC 2006). Flavonoid was determined by gravimetric oven drying method as described by Bohm and Kocipai-Abyazan (1994).

Statistical Analysis

All determinations were done in duplicates. The data generated were entered into the computer and analyzed using Statistical Package for Social Sciences (SPSS version 16.0) Means and standard deviation obtained from the chemical analysis were calculated. Analysis of variance (ANOVA) was used to compare the values

Table 1. Effect of soaking time on the energy and proximate composition of bambara groundnut(*Vigna subterranean*)

Nutrient	Un-soaked BGN	BGN soaked for 6hr	BGN soaked for 24hr	BGN soaked for 48hr	BGN soaked for 72hr
Moisture (g/100g)	10.2^{c}± 0.06	10.3^{b}± 0.04	10.4^{b}± 0.03	11.2^{a}± 0.07	11.2^{a} ±0.03
protein (g/100g)	22.4^{a} ±0.06	22.3^{ab}±0.02	22.2^{ab}±0.00	22.1^{b}±0.04	20.2^{a}±0.03
Fiber(g/100g)	4.8 ^{a}±0.02	4.4^{b} ±0.04	4.2^{c} ±0.01	4.2 ^{d}±0.04	4.1^{e} ±0.01
Fat (g/100g)	7.2^{a} ±0.08	6.2^{b} ±0.11	6.1^{c} ±0.01	5.9 ^{d}±0.03	5.9^{e} ±0.00
Ash(g/100g)	3.9^{a}±0.01	3.8b± 0.88	3.7^{c}±0.01	3.7^{d}±0.01	3.6^{e} ±0.04
CHO (g/100g)	51^{d}	52^{c}	53^{b}	52 ^{c}	55^{a}
Energy (kcal/kj)	360/1524	356/1506	356/1507	352/1492	350/1478

Values with the same superscript on the same roll are not significantly different (p>0.05) from each other. BGN- Bambara ground nut

Table 2. Effect of soaking time on the mineral composition of bambara groundnut(*Vigna subterranean*)

Nutrient	Un-soaked BGN	BGN soaked for 6hr	BGN soaked for 24hr	BGN soaked for 48hr	BGN soaked for 72hr
Ca(mg/100g)	387.4^{a} ±0.02	376 5^{b}± 0.08	365.8^{c}± 0.02	358.5^{d} ±0.09	339.6^{e}± 0.22
Mg(mg/100g)	192.4^{a} ±0.07	189.4^{b}± 0.04	128.6^{c} ±0.01	125.3^{d} ±0.04	122.8^{e} ±0.20
Na(mg/100g)	11.7^{a} ±0.03	11.2^{b} ±0.09	10.8^{c} ±0.04	10.2^{d} ±0.02	9.8^{e} ±0.11
K(mg/100g)	35.6^{a} ±0.21	34.7^{b} ±0.03	32.8^{c} ±0.14	31.6^{d}± 0.10	31.6^{d}± 0.18
Fe(mg/100g)	1.8^{a} ±0.07	1.6^{b} ±0.01	1.5^{c} ±0.08	1.4^{d} ±0.01	1.4^{d} ±0.03
Cu(mg/100g)	0.21^{a} ±0.01	0.16^{a} ±0.00	0.13^{c} ±0.04	0.09^{d} ±0.01	0.02^{e} ±0.00
Se(mg/100g)	0.14^{a} ±0.02	0.12^{b} ±0.01	0.08^{c} ±0.00	0.07^{cd} ±0.03	0.05^{d} ±0.00
I(mcg/100g)	24.8^{a} ±0.04	21.6^{b}± 0.02	18.7^{c} ±0.02	16.6^{d}± 0.04	13.8^{e} ±0.08

Values with the same superscript on the same roll are not significantly different (p>0.05) from each other. BGN- Bambara ground nut.

Table 3. Effect of soaking time on the vitamin composition of bambara groundnut(*Vigna subterranean*)

Nutrient	Un-soaked BGN	BGN soaked for 6hr	BGN soaked for 24hr	BGN soaked for 48hr	BGNsoaked for 72hr
B-carotene (mcg/100g)	8.83^{a} ±0.00	7.24^{b} ±0.01	6.35^{c} ±0.12	5.75^{d}± 0.06	5.22^{e}±0.02
Vitamin C (mg/100g)	1.79^{a} ±0.00	1.66^{b} ±0.01	1.42^{c} ±0.04	1.29^{d} ±0.04	1.06^{e} ±0.01
Vitamin E (mg/100g)	0.85^{a} ±0.02	0.76^{b} ±0.00	1.73^{b} ±0.02	0.68^{c} ±0.02	0.59^{d} ±0.00
Vitamin B_1 (mg/100g)	0.40^{a} ±0.01	0.28^{b} ±0.01	0.21^{c} ±0.03	0.16^{d} ±0.00	0.13^{e} ±0.00
Vitamin B_2 (mg/100g)	0.15^{a} ±0.02	0.12^{b} ±0.00	0.09^{c} ±0.00	0.08^{c}±0.00	0.06^{d} ±0.01
Vitamin B_3(mg/100g)	2.34^{a}± 0.02	2.17^{b} ±0.00	1.87^{c} ±0.00	1.72^{d} ±0.01	1.59^{d} ±0.02

Values with the same superscript on the same roll are not significantly different (p>0.05) from each other. BGN- Bambara ground nut.

obtained the tests. Duncan Multiple New range test was used to separate means and significance accepted at p<0.05.

RESULTS AND DISCUSSION

The effect of soaking time on the proximate composition of bambara groundnut is shown on Table 1. The result showed that soaking bambara groundnut seeds for varying periods increased the percentage moisture. The moisture content of the samples ranged between 10.2 - 11.2% with the un-soaked bambara groundnut having the lowest moisture value (10.2%) while bambara groundnut soaked for 48hr and 72hr had the highest moisture value (11.2%). Unlike the report given by Adebayo (2014) who observed significant difference in the moisture content of all the samples studied, the moisture content of samples soaked for 6hr and the one soaked for 24hrs in this study were not significantly different from each other. The same observation was made for samples soaked for 48hrs and 72hrs. The high

Table 4. Effect of soaking time on the anti-nutrient composition of bambara groundnut(*Vigna subterranean*)

Nutrient	Un-soaked BGN	BGN soaked for 6hr	BGN soaked for 24hr	BGN soaked for 48hr	BGN soaked for 72hr
Phytate	4.54^{a}± 0.00	2.79^{b} ±0.00	1.65^{c}± 0.01	0.94^{d} ±0.00	0.35^{e}± 0.01
Alkaloid	0.78^{a}± 0.01	0.46^{b} ±0.00	0.33^{c} ±0.01	0.29^{d} ±0.00	0.18^{e} ±0.00
Tannin	0.45^{a}±0.00	0.28^{b} ±0.01	0.19^{c} ±0.00	0.16^{d} ±0.01	0.11^{e} ±0.00
Oxalate	1.06^{a} ±0.00	0.77^{b} ±0.01	0.52^{c} ±0.01	0.38^{d} ±0.01	0.29^{e} ±0.01
Saponin	0.82^{a} ±0.01	0.68^{b} ±0.00	0.45^{c} ±0.02	0.24^{d} ±0.01	0.12^{e} ±0.01
HCN	5.79^{a} ±0.05	4.17^{b} ±0.03	3.24^{c} ±0.03	1.75^{d} ±0.02	0.46^{e} ±0.02
Trypsin	12.62^{a}± 0.02	8.37^{b}± 0.02	5.78^{c} ±0.03	3.81^{d}± 0.01	1.46^{e} ±0.02

Values with the same superscript on the same roll are not significantly different (p>0.05) from each other. BGN- Bambara ground nut; HCN- Hydrogen cyanide

moisture observed with increasing soaking time implies that the retention of residual moisture in the sample is relative to soaking time. Increase in percentage moisture in relation to soaking time was reported in a similar work carried out on sorghum (Obizoba and Atii, 1991) and lima bean (Adebayo, 2014) respectively. The result of the effect of soaking time on protein, crude fiber, crude fat and ash showed that the values of protein (20.2-22.4%), crude fiber (4.1- 4.8%), crude fat (5.9-7.2%), and ash (3.6-3.9%) decreased with increasing soaking time. This finding was not surprising because it is a known fact that the nutrient composition of any food is a function of its moisture content. Obasi and Wogu (2008) reported similar finding in a work they carried out on maize. Adebayo (2014), however reported increase in protein, ash, fat content of lima bean that were soaked for 12hr, 36hr and 24hr respectively.

The increase in protein, ash and in fat in that study was attributed to the effect of fermentation. The carbohydrate and energy values of the samples ranged between 51.6-55.3% and 1478-1524kj respectively. The energy values of all the samples also decreased with soaking time. The energy value any food is a function of its protein, fat and carbohydrate function.

The mineral compositions of the samples are shown on Table 2. The result shows decrease of 2.85%- 12.4% for calcium, 1.54%-36.2% for magnesium, 4.27%-16.2% for sodium, 2.24%-11.2% for potassium, 6.21%-22.0% for iron, 23.8%-90.0% for copper, 14.28%- 64.28% for selenium and 12.9%-44.5% for iodine respectively. This finding implies that the mineral contents of the samples decreased as the soaking time progressed; and the decrease in minerals could be attributable to the effect of leaching. Though the values of Ca and Fe like those of the other minerals reduced with soaking time, research however showed that soaking and germination improves their bioavailability (Beruk, 2015).

The effect of soaking time on the vitamin composition of bambara groundnut is shown Table 3. There was significant decrease in all the vitamins except for vitamin B_2 in the sample soaked for 24hr and in the sample soaked for 48hr (0.09mg/100g, 0.08mg/100g respectively). Decrease in vitamin content due to effect of soaking time was expected because it has been shown that vitamins (particularly the water soluble vitamin) are water labile.

The effect of soaking time on the anti-nutrient composition of bambara groundnut on Table 4 shows reduction in phytate (4.54 -0.35mg/100g), alkaloid (0.78-0.18mg/100g), tannin (0.45-0.11mg/100g), oxalate (1.06-0.29mg/100g), saponin (0.82-0.12mg/100g), HCN (5.79 - 0.46mg/100g) and trypsin (12.62- 1.46mg/100g) respectively. The reduction observed in all the anti-nutrient content of the samples might be as a result of leaching. Reduction in anti-nutrient value with respect to soaking time was also reported by Adebayo (2014) in a work he carried out on lima beans. It is noteworthy to state that all the anti-nutrient composition of un-soaked and soaked bambara groundnut were within permissible limit (Anigo *et al*., 2010). This implies that soaking bambara groundnut for ≤6hr is recommended for maximum nutrient retention.

CONCLUSION

The finding showed increase of residual moisture in all the samples. Decrease in nutrients (protein, fat, fiber, carbohydrate, minerals and vitamins) and in anti-nutrients (phytate, alkaloid, tannin, oxalate, saponin, HCN and trypsin) were observed throughout the soaking period. It was found that all the anti-nutrient composition of un-soaked and soaked bambara groundnut were within permissible limit which implies that soaking bambara groundnut for ≤6hr will be recommended for maximum nutrient retention.

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