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Effect of Soaking and Boiling on Anti-nutritional Factors, Oligosaccharide Contents and Protein Digestibility of Newly Developed Bambara Groundnut Cultivars

Olaposi Rafiu Adeleke[1*], Oladipupo Qudus Adiamo[2], Olumide Samson Fawale[1], Gbeminiyi Olamiti[3]

[1]*Department of Food Science and Technology, Faculty of Technology, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria*
[2]*Department of Food Science and Nutrition, College of Food and Agricultural Sciences, King Saud University, P.O. Box 2460, Riyadh 11451, Kingdom of Saudi Arabia*
[3]*Department of Food Science and Technology, School of Agriculture, University of Venda, Thohoyandou 0950, South Africa*

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[*]Corresponding Author:
E-mail: adeleke.olaposi@yahoo.com

ABSTRACT

Newly developed Bambara groundnut (*Vigna subterranea* L.) seeds (Accessions No: TVSU 5 – Bambara Groundnut White (BGW) and TVSU 146 – Bambara Groundnut Brown (BGB)) were collected from International Institute of Tropical Agriculture (IITA), Nigeria, planted and harvested. The effects of processing methods (soaking and boiling) on anti-nutritional factors and oligosaccharides content and protein digestibility of BGW and BGB compared with Bambara groundnut commercial (BGC) seeds were investigated. Soaking and boiling significantly reduced the anti-nutritional factors of the samples and the effect increased as processing time was elongated. Sample BGC had lower anti-nutritional factors than BGW and BGB after soaking for 48 h. Tannin contents of the samples were reduced drastically by 99 % throughout the soaking periods. Greatest loss in raffinose level was observed in BGB (59%) and BGW (50%) after boiling for 60 min compared with BGC (43%). The loss in stachyose content of the samples varies with processing and BGC (59%) had greatest loss after boiling for 60 min while soaking for 48 h reduced that of BGB and BGW by 57 and 35%, respectively. Boiling for 60 min increased the *in vitro* protein digestibility of BGB (89.34 %) compared with BGW (87.48%) and BGC (82.89%). Overall, the results demonstrated that soaking and boiling of newly developed Bambara groundnut seeds could improve the nutritive quality of the seeds.

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Introduction

A significant part of the human world population relies on legumes as their main source of proteins. Nonetheless, production of common legumes may not keep pace with population growth due to adverse weather conditions and consequently decline per head capital availability. In rural South Western Nigeria, legumes are often advocated to be included in diets by Nigerian Nutrition Society and National Committee on Food and Nutrition (NCFN) due to their high nutritional values and low cost (Akinjayeju and Bisiriyu, 2004). Intensive efforts to find alternative sources of proteins from plants adapted to adverse conditions have been conducted (Adebowale and Lawal, 2003; Akinjayeju and Bisiriyu, 2004) with little success. However, most of the indigenous legumes in Nigeria and in the tropics are relatively underutilized (Bryan, 2000).

Bambara groundnut (*Vigna subterranea* L.) is one of the indigenous underutilized legumes that are now grown across Africa (Adebowale and Lawal, 2003). It is an herbaceous, intermediate, annual plant, with creeping stems at ground level. Bambara groundnut can grow under conditions unsuitable for groundnut and had tolerance for drought and poor soil (Agbenorhevi et al., 2007). The seed contains about 19.60-21.42% crude protein and about 3.33-6.50% crude fat (Fadahunsi and Sanni, 2010; Fadahunsi et al., 2011; Okonkwo and Opara, 2010). It is usually fried or boiled with salt and eaten as snacks or pounded into flour and used in the preparation of soup, porridge and various fried or steamed food products such as akara, moin-moin and okpa in Nigeria. Adebowale et al. (2005) reported that bambara groundnut flour has been used in making bread in Zambia. Piyarat (2008) noted that the milk prepared from bambara groundnut gave a preferred flavour to that of milks from cowpea, pigeon pea and soybean.

Some of the factors that limit the utilization potential of indigenous legumes include presence of anti-nutrients (trypsin, chymotrypsin, α-amylase inhibitors, phytic acid,

flatus factors, saponin and lectins), low contents of sulphur amino acids and the long cooking period required to eliminate those anti-nutritional factors (Genta et al., 2002). Furthermore, the presence of indigestible substances such as the flatulence-producing oligosaccharides, namely raffinose, stachyose and verbascose has also contributed to their reduced utilization as human foods (Aremu et al., 2006).

The nutritive value of legumes depends upon the processing methods, presence or absence of antinutritional or toxic factors and possible interaction of nutrient with other food component (Ghadge et al., 2008). The level of anti-nutritional factors in legumes need to be reduced in order to improve the nutritional quality and enhance effective utilization of the legume for human consumption. So it is necessary to establish processing techniques to ensure its optimal utilization. In order to inactivate or reduce anti-nutrients, various conventional, simple processing methods have been used in legume seeds (Barbour et al., 2001; Farran et al., 2001).

Heat processing is widely accepted as an effective means of inactivating the thermo-labile antinutritional factors of legume grains. Cooking, autoclaving, pressure cooking, extrusion cooking and microwave treatment are some of the heat processing treatments commonly applied to legume grains before their consumption (Osungbade et al., 2016; El-Adawy, 2002; Khokhar and Chauhan, 1997; Alonso et al., 2000). They reported that these improved protein quality by inactivating anti-physiological factors particularly trypsin inhibitors and haemagglutinins by unfolding the protein structure, thus making them more susceptible to attack by digestive enzymes. Moist heat has been reported to be more effective than dry heat and the degree of inactivation is governed by temperature, duration of heating and particle size. Other treatments reported to have reduced the undesirable constituents of legume grains include soaking (Khokhar and Chauhan, 1997), germination (Wang et al., 2009), irradiation (Ghadge et al., 2008) as well as chemical treatments (Fernandez et al., 1993).

In spite of the growing importance of bambara groundnut, research efforts in Nigeria have been concentrated on its agronomy. Little or no attention has been paid to the technologies for its processing. Furthermore, much of the available data and information on the anti-nutrient composition are limited to the commonly used variety. This is because of the possible effects of variety/genetic origin, climate, soil, pesticides and fertilizers on the chemical composition of the new varieties. Therefore, the aim of this study was to determine the effects of soaking and boiling on anti nutritional factors and oligosaccharides contents and protein digestibility of newly developed Bambara groundnut cultivars.

Materials and Methods

Collection of Raw Materials

Figure 1 shows the new varieties of Bambara groundnut seeds and the commercial sample used in this study. Two new varieties of bambara groundnut (*Vigna subterranea* L.) seeds (Accessions No: TVSU 5 – Bambara Groundnut White (BGW) and TVSU 146 – Bambara Groundnut Brown (BGB) were collected from the International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State, Nigeria. The seeds were multiplied by planting on a special and demarcated farm at Kelebe, Osogbo, Osun State, Nigeria. The ripe, matured and dried pods were harvested after 160 days. The pods were sun dried and threshed to obtain the seeds. Bambara groundnut commercial (BGC) seeds were purchased from a local market in Oyo, Nigeria. All the seeds were washed with clean tap water to remove dirt and other adhering materials, sun dried and stored in air tight containers for analysis. All chemicals used were of analytical grade.

Preparation of Raw, Soaked and Boiled Bambara Groundnut Seeds Flour

Raw flour was prepared by steeping 500 g of bambara groundnut seeds in distilled water for 30 min, decorticated by rubbing between palms and the seed coats were washed away with tap water. Soaking was done according to the method described by Udensi et al. (2008) with slight modification. The seeds were soaked in distilled water (1:10 w/v) at room temperature (30±2ºC) for 12, 24 and 48 h thereafter the soaked seeds were washed twice with ordinary water, followed by rinsing with distilled water, drained and dehulled. Boiling was carried out by heating the seeds in distilled water at 100ºC (sample: water ratio; 1:10) for 30 and 60 min, drained and dehulled. All the treated samples were dried at 40ºC in a hot air oven (UNISCOPE SM 9053, England) to about 10% moisture content. The dried samples were milled using Marlex Excella grinder (Marlex Appliances PVT, Daman) and sieved through 0.2 mm sieve to obtain homogenous flour that were kept in air tight containers and refrigerated at 4°C for further analyses.

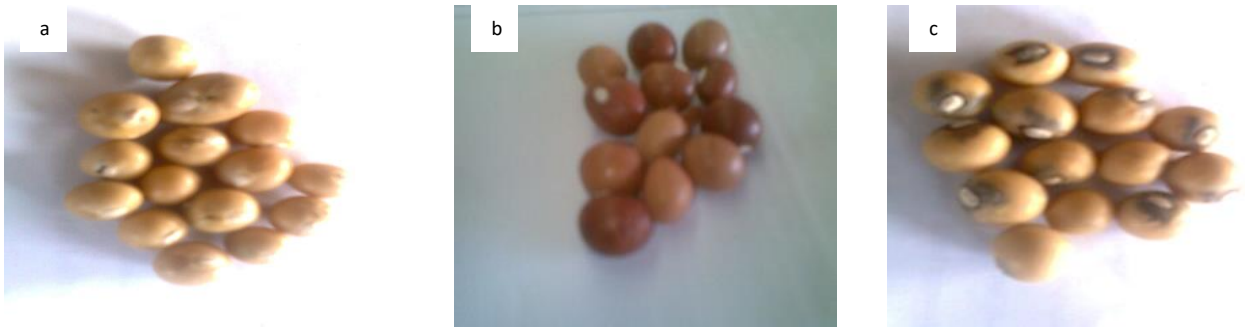

Figure1 Varieties of Bambara groundnut seeds a) Accession No: TVSU-5 variety (Bambara Groundnut White -BGW), b) Accession No: TVSU-146 variety (Bambara Groundnut Brown- BGB), c) Bambara Groundnut Commercial- BGC

Oxalate Level Determination

Oxalate was determined by the method described by Falade et al. (2005) with slight modification. Two grams of the sample was extracted with 190 mL of distilled water and 10 mL of 6 M HCl in boiling water for 2 h, filtered and made up to 250 mL with water. An aliquot (50 mL) of the filtrate was titrated against NH_4OH until the salmon pink of the methyl red indicator changed to a faint yellow. The solution was heated to 90°C and 10 mL of 5% (w/v) $CaCl_2$ solution was added to precipitate the oxalate overnight. The precipitate was washed free of calcium and then washed into a 100 mL conical flask with 10 mL of hot H_2SO_4 (25% v/v) and then with 15 mL distilled water. The final solution was heated to 90°C and titrated against a standardized 0.1 M $KMNO_4$ until a faint purple color of the solution persisted for 30 s. The oxalate was then calculated as the sodium oxalate equivalent from the mole ratio.

Tannin Content Determination

Vanillin-HCl method was used to assay tannin content according to the modified method of Price and Butler (1977). Briefly, extraction was done by adding 1 g of sample to 50 mL methanol. The mixture was stirred for 20-28 h and centrifuged to obtain the supernatant. Exactly 1 mL of supernatant was added to 5 mL of vanillin hydrochloric acid reagent (equal volumes of 8% hydrochloric acid in methanol and 4% vanillin in methanol). A standard catechin solution (20-100 µg) was prepared and used as reference standard. The absorbance of sample and standard solutions were read after 20 min in Jenway V6300 spectrophotometer at 500 nm.

Phytate Content Determination

Phytate content was determined by the method described by Wheeler and Ferrel (1971) using 2.0 g dried sample. A standard curve was prepared expressing the results as $Fe(NO_3)_3$ equivalent. Phytate phosphorus was calculated from the standard curve assuming a 4:6 iron to phosphorus molar ratio.

Saponin Content Determination

The spectrophotometric method of Brunner (1984) was used for saponin analysis using 1g of sample. Standard saponin solutions (0–10 ppm) were prepared and used as reference standard. The absorbance of sample and standard solutions were read after colour development in a Jenway V6300 spectrophotometer at a wavelength of 380 nm.

Trypsin Inhibitors Determination

This was determined by the method of Mbata et al. (2009) with slight modification. Approximately, 1.0 g portion of the sample was extracted by soaking overnight at 4°C in 50 mL of 0.01 M NaOH; pH was adjusted to 8.4-10.0. The suspension was diluted so that 2 mL of the sample extract inhibited 40-60% of standard trypsin used in the analysis. Synthetic benzoyl-dl-arginine-p-nitroamide was used as substrate for the inhibition of trypsin. A residual enzyme activity was determined in 2 mL aliquot of the sample extracts by measuring at 410 nm. Trypsin inhibitor activity (TIA) in term of milligrams pure trypsin inhibited per sample (g) was calculated as:

$$\text{TIA} = \frac{(2.632 \times \text{D} \times \text{A}_1)}{\text{S (mg) pure trypsin inhibited g}^{-1}\text{ sample}}$$

Where A_1 = change in absorbance due to trypsin inhibition/mL diluted sample extract, D =dilution factor and S = weight of sample (g)

Oligosaccharides Content Determination

Five g of sample were added to 50 mL of 70% ethanol (v/v) and stirred for 12 h. The content of the flask was filtered through Whatman No. 1 filter paper and the residue was further washed with 25 mL of 70% ethanol. The combined filtrate was evaporated in a rotary vacuum evaporator at 40ºC, freeze dried and re-suspended in 10 mL of distilled water. Ten microliters of the above syrup were spotted in triplicate on chromatographic plates (19 × 19 cm) coated with cellulose Powder-G. The plates were kept in a chromatographic chamber containing *n*-propanol: ethyl acetate: water (6:1:3) as the solvent system (Tanaka et al., 1975). The developed plates were sprayed with 1% α-naphthol in ethyl alcohol containing 10% orthophosphoric acid to locate the sugar spots. For quantitative estimation, the area (2x3 cm) corresponding to each oligosaccharide was scraped and soaked in 2 mL of distilled water kept overnight and filtered through Whatman No. 1 filter paper. The eluted individual oligosaccharides (raffinose and stachyose) were estimated by the method of Tanaka et al. (1975).

In vitro Protein Digestibility Determination

In vitro protein digestibility of 60 min-boiled samples and 48 h-soaked samples were measured according to the method descried by Saunders et al. (1973), using pepsin and pancreatin digestion method. The digested protein was analyzed for nitrogen using micro Kjeldahl procedure (AOAC, 2005) and expressed as a percent of the total N.

Statistical Analysis

All experiments were carried out in triplicate, and data were assessed using ANOVA described by Snedecor and Cochran (1987). Differences between the treatment means were separated using Duncan's multiple range tests. Significance was accepted at $P<0.05$.

Results and Discussion

Effect of Soaking on Antinutrients Contents of BGC, BGB and BGW

The effect of soaking for 12, 24 and 48 h on the antinutrients contents of the samples is shown in Table 1. Raw BGB (7.49 mg/100g) had the lowest amount of saponin while BGW (9.10 mg/100g) had the highest value. A decrease ($P<0.05$) in saponin level with increase in soaking time in all the samples was observed. The saponin content reduced significantly ($P<0.05$) after soaking for 48 h by 64, 49 and 11% for BGC, BGW and

BGB, respectively. After soaking for 48 h, sample BGB was reduced to 6.65 mg/100g while BGW had 4.62 mg/100g. The saponin levels reported in this study were higher than 1.37 mg/100g reported by Mbagwu et al. (2011) for bambara groundnut after soaking for 48 h. The difference could be attributed to differences in seed coat permeability of the samples which affects the rate of leaching. Saponins are characterized by a bitter taste and foaming properties. The adverse effect of saponins can be overcome by repeated washing with water which makes the food more palatable by reducing the bitterness associated with it (Joshi et al., 2009).

Table 1 Effect of soaking on the antinutrients contents of Bambara groundnut seeds

Sample	Time	Saponin		Tannin		Phytate		Oxalate		Trypsin inhibitor	
		Total (mg/100g)	RD (%)	Total (mg/100g)	RD (%)	Total (mg/100g)	RD (%)	Total (mg/100g)	RD (%)	Total (Tiu/100g)	RD (%)
BGC	0	8.40±0.01^{a}	0	1.65±0.01^{a}	-	850±0.32^{a}	-	0.39±0.07^{a}	-	17.56±0.40^{a}	-
	12 h	7.01±0.14^{b}	17	0.01±0.00^{b}	99	590±0.09^{b}	31	0.19±0.03^{b}	51	11.67±0.21^{b}	34
	24 h	6.40±0.05^{c}	23	0.01±0.00^{b}	99	380±0.03^{c}	55	0.11±0.01^{b}	72	10.46±0.11^{c}	40
	48 h	3.04±0.22^{d}	64	0.01±0.00^{b}	99	270±0.01^{d}	68	0.01±0.03^{c}	97	10.01±0.12^{c}	43
BGW	0	9.10±0.04^{a}	-	0.75±0.04^{a}	-	813±1.42^{a}	-	0.26±0.01^{a}	-	13.15±0.03^{a}	-
	12 h	7.37±0.09^{b}	19	0.01±0.00^{b}	99	430±0.04^{b}	47	0.14±0.04^{b}	46	9.05±0.35^{b}	31
	24 h	6.81±0.11^{c}	25	0.00±0.00^{b}	99	320±0.12^{c}	61	0.12±0.06^{c}	54	8.99±0.14^{b}	32
	48 h	4.62±0.05^{d}	49	0.00±0.00^{b}	99	297±0.04^{d}	63	0.09±0.07^{d}	65	8.76±0.03^{b}	33
BGB	0	7.49±0.32^{a}	-	1.00±0.06^{a}	-	970±0.74^{a}	-	0.30±0.07^{a}	-	16.15±0.57^{a}	-
	12 h	7.32±0.06^{b}	2	0.01±0.00^{b}	99	744±0.36^{b}	23	0.21±0.01^{b}	30	13.15±0.56^{b}	19
	24 h	7.04±0.18^{c}	6	0.01±0.00^{b}	99	562±0.02^{c}	42	0.17±0.04^{c}	43	10.26±0.34^{c}	36
	48 h	6.65±0.07^{d}	11	0.01±0.00^{b}	99	437±0.13^{d}	55	0.10±0.08^{d}	67	9.89±0.56^{c}	39

RD: Reduction, Values are means ± standard deviation of three determinations. Means in a column not sharing a common superscript letter are significantly (P<0.05) different as assessed by LSD test. Bambara groundnut white (BGW); Bambara groundnut brown (BGB); Bambara groundnut commercial (BGC)

Highest tannin content was found in raw BGC (1.65 mg/100g) while BGW (0.75 mg/100g) had the lowest value. After soaking for 12 h, 99% reduction in tannin contents of all samples was observed (P<0.05). The significant reduction in tannin level in soaked samples may be due to removal of seed coat and leaching of tannin (a water soluble phenolic compounds) from the cells into water. The values obtained were lower than the results reported by Udensi et al. (2008) and Onwurafor et al. (2013) where the tannin content of bambara groundnut seeds was investigated. Mazahib et al. (2013) observed that soaking reduced the tannin content of some cowpea varieties, African yam beans, bambara groundnut, and hyacinth and rajina beans to non-detectable levels. Tannins are water soluble phenolic compounds with a high molecular weight and with the ability to form complexes which are not readily digestible. Tannins could reduce availability of proteins because it could complex it and render it unavailable. Tannin is generally present in the seed coat and its removal should eliminate this toxic constituent and improve the nutritive value of food products.

The phytate content of the raw samples ranged between 850 mg/100g in BGC to 970 mg/100g in BGB. Soaking significantly (P<0.05) reduced the phytate content compared to raw samples with greater effect observed as soaking time was elongated. After 48 h of soaking, the phytate content in BGW and BGB was reduced by 63 and 55% compared with that of BGC (68%). The reduction in phytate level after soaking for various times agreed with the result reported by Udensi et al. (2008) where the phytate level of *Mucuna flagellipes* reduced from 0.387 to 0.097 (74.9% reduction) after soaking for 24 h. Bambara groundnut seeds are rich in protein (18.50-20.73%), therefore, they had high phytate levels (Mazahib et al., 2013). In legumes, phytate are associated with protein bodies (Suleiman et al., 2007) and therefore phytate levels should increase with increasing protein content. Phytic acids form insoluble salts with essential minerals like calcium, iron, magnesium and zinc in food rendering them unavailable for absorption into the blood stream.

Highest amount of oxalate was observed in BGC (0.39 mg/100g) while BGW and BGB had 0.26 and 0.30 mg/100g, respectively. Soaking significantly (P<0.05) reduced the oxalate content of all the samples and this decreased as soaking time increased. After 48 h of soaking, a significant reduction (97%) of oxalate level was observed in BGC compared with BGW (65%) and BGB (67%). Obasi and Nwogu (2008) reported that soaking caused a significant reduction in the level of oxalate in cowpea. Oxalate combines with calcium to form calcium oxalate which passes through the intestine without being absorbed. Calcium oxalate is responsible for most of the kidney stone formation. Formation of these stones frequently reflects chronic alkalinity of bladder and renal pelvic urine caused by inflectional bacterial that hydrolyses urea, releasing ammonia (Olaleye et al., 2013).

The trypsin inhibitors contents of the samples ranged between 13.15 to 17.56 Tiu/100g with BGW having the lowest value. The levels of trypsin inhibitors after soaking reduced (P<0.05) with increase in soaking time. Soaking for 48 h significantly (P<0.05) reduced the level of trypsin inhibitors in BGW to 8.76 Tiu/100g while highest level was recorded in BGC (10.01 Tiu/100g). The trypsin inhibitor levels after soaking for 48 h were still higher

than the values of 4.28 Tiu/100g reported by Fadahunsi and Sanni (2010) for soaked bambara groundnut seeds.

Effect of Boiling on Antinutrients Contents of BGC, BGB and BGW

The effect of boiling for different periods on the antinutrients contents of BGC, BGB and BGW is shown in Table 2. It was observed that the saponins content of raw BGC was found to be 8.40 mg/100g while that of BGW and BGB were 9.10 and 7.49 mg/100g, respectively. Boiling for 30 min significantly ($P<0.05$) reduced the levels of saponin in BGC (by 28 %), BGW (by 11%) and BGB (by 5%). Increasing the boiling time to 60 min significantly ($P<0.05$) reduced the levels of saponin of BGC to 5.32 while that of BGW and BGB were reduced to 6.01 and 6.78 mg/100g, respectively. The values were higher than that of bambara groundnut (0.43 mg/100g), cowpea (0.25 mg/100g) and soybean (0.39 mg/100g) reported by Mbagwu et al. (2011). However, the values were lower than that of whole and defatted bambara groundnut seeds, 13.8 and 10.1 mg/100g of saponin, respectively, reported by Olaleye et al. (2013).

Raw BGC (1.65 mg/100g) had the highest tannin contents while BGW (0.75 mg/100g) had the lowest value. Significant ($P<0.05$) reduction in tannin contents of BGW and BGB by 49 and 31% was observed after boiling for 60 min while BGC was reduced by 60%. The reduction in tannin level with cooking time is in agreement with the report of Adewusi and Osuntokun (1989) that tannin is possible culprit in the hard-to-cook phenomenon of some beans and that it could play a contributory role. Mazahib et al. (2013) reported that tannin content of bambara groundnut was found to be 4.60 mg/100g while Abiodun and Adepeju (2011) reported 2.40 mg/100g for bambara seed flour. The results of these findings were in disagreement with earlier observation by Adewusi and Osuntogun (1989) that boiling did not appreciably affect the tannin content of bambara groundnut and lima beans.

The level of phytate in raw samples BGC (850 mg/100g) was higher than that of BGW (813 mg/100g) and BGB (610 mg/100g). The phytate content of the samples was significantly ($P<0.05$) reduced with increase in boiling time. After boiling for 60 min, greater reduction was observed in BGB (46 %) while BGC and BGW were reduced by 19 and 23%, respectively. This result agrees with that reported by Mazahib et al. (2013) that boiling reduced the level of phytate but the reduction obtained in this study was greater than that obtained when raw bambara groundnut seeds was boiled for the same period (Mazahib et al. 2013). The values were also lower than that of boiled and whole bambara groundnut seeds were 14.4 and 17.5 mg/100g, respectively as reported by Olaleye et al. (2013). According to El Maki et al. (2007), the difference in the loss of phytic acid during boiling could probably be explained on the basis that phytate activity at a temperature of 40-55°C may degrade inositol hexaphosphate to the pentaphosphate or lower molecular weight forms. It was also reported that phytic acid content decreased during boiling because insoluble complexes between phytate and other compounds were formed and accordingly the amount of free phytate will be reduced (Mazahib et al., 2013).

Raw BGC had the highest oxalate content (0.39 mg/100g), while BGW had the lowest (0.26 mg/100g). The oxalate content of the raw bambara groundnut seeds decreased ($P<0.05$) with increase in boiling time. After 30 min of boiling, the levels of oxalate in BGC, BGW and BGB were decreased to 0.13 and 0.21 mg/100g, respectively, compared with that of BGC (0.17 mg/100g). Increase in boiling time to 60 min did not have any significant effect on the level of oxalate in the BGW (58%) and BGB (34%) but significantly ($P<0.05$) reduced that of BGC by 67%. The decreases in oxalate content of the samples after boiling were in conformity with the results reported by Ijarotimi et al. (2009) and Olaleye et al. (2013) that boiling reduced the oxalate content of bambara groundnut seeds.

The trypsin inhibitor (TI) of BGC, BGW and BGB are 17.56, 13.15 and 16.15 Tiu/100g, respectively. The activity of TI in the samples boiled for different times showed significant decrease ($P<0.05$) when compared with that of raw samples with greater effect observed as boiling time increased. After 60 min of boiling, BGC (67%) exhibited higher reduction in TI levels than that of BGW (40%) and BGB (43%). Boiling significantly affected TI activity and the inhibition effect depends on time of cooking. This is in line with the report of Adeyeye (2001) who stated that reduction in trypsin inhibitors activity occurred mainly during the first step of cooking for both early and hard-to-cook beans. However, apparent retention of trypsin inhibitory activity was reduced to about 50% by applying heat treatments till beans were softened. Thus, the residual trypsin inhibitors will be resistant, since heat treatment must have deactivated the heat labile trypsin inhibitors. The non-protein trypsin inhibitory activity was reported to compose 27 to 55% of the total trypsin inhibitory activity in soybean and 4 to 15% of the total activity of winged bean (Apata and Ologhobo, 1997). Adewusi et al. (2008) reported that boiling reduced the level of trypsin inhibitors after 60 and 90 min by 49.1 and 50.1% in *Mucuna flagellipes*. It can be concluded from the results that boiling can reduce to a significant level the trypsin inhibitors in the newly developed bambara groundnut seeds cultivars.

Effect of Processing Treatments on Oligosaccharides Content of BGC, BGW and BGB

The levels of raffinose and stachyose in the raw and processed BGC, BGW and BGB are presented in Table 3. Raw BGW had lower level of raffinose (1.30 g/100g) and stachyose (0.86 g/100g) than that of BGB (1.79 and 1.22 g/100g, respectively) and BGC (2.05 and 1.48 g/100g, respectively). The concentrations of raffinose in the samples were higher than that of red bean (0.89 g/100g) and chick pea (1.00 g/100g) reported by Ruiz-Teran and Owens (1996). The levels of stachyose in the samples were lower than that of soybeans (2.08 g/100g) reported by Mercedes et al. (1999).

Table 2 Effect of boiling on the antinutrients content of Bambara groundnut seeds

Sample	Time	Saponin		Tannin		Phytate		Oxalate		Trypsin inhibitor	
		Total (mg/100g)	RD (%)	Total (mg/100g)	RD (%)	Total (mg/100g)	RD (%)	Total (mg/100g)	RD (%)	Total (Tiu/100g)	RD (%)
BGC	0	8.40±0.04^{a}	-	1.65±0.01^{a}	-	850±0.32^{a}	-	0.39±0.01^{a}	-	17.56±1.25^{a}	-
	30min	6.04±0.27^{b}	28	0.96±0.01^{b}	42	799±13.02^{b}	6	0.17±0.04^{b}	56	7.26±0.10^{b}	59
	60min	5.32±0.15^{c}	37	0.66±0.01^{c}	60	685±17.00^{c}	19	0.13±0.03^{c}	67	5.74±0.14^{c}	67
BGW	0	9.10±0.04^{a}	-	0.75±0.04^{a}	-	813± 1.42^{a}	-	0.26±0.01^{a}	-	13.15±0.03^{a}	-
	30min	8.11±0.03^{b}	11	0.41±0.01^{b}	45	645±3.79^{b}	21	0.13±0.04^{b}	50	8.34±0.45^{b}	37
	60min	6.01±0.07^{c}	34	0.38±0.00^{c}	49	630±3.11^{c}	23	0.11±0.06^{c}	58	7.88±0.34^{b}	40
BGB	0	7.49±0.01^{a}	-	1.00±0.06^{a}	-	670±7.70^{a}	-	0.29±0.10^{a}	-	16.15±0.07^{a}	-
	30min	7.14±0.02^{b}	5	0.82±0.03^{b}	18	610±3.40^{b}	9	0.21±0.01^{b}	28	10.23±0.06^{b}	37
	60min	6.78±0.01^{c}	9	0.69±0.02^{c}	31	365±4.50^{c}	46	0.19±0.04^{b}	34	9.15±0.01^{c}	43

RD: Reduction, Values are means ± standard deviation of three determinations. Means in a column not sharing a common superscript letter are significantly (P<0.05) different as assessed by LSD test. Bambara groundnut white (BGW); Bambara groundnut brown (BGB); Bambara groundnut commercial (BGC)

Table 3 Effect of soaking and boiling on oligosaccharides content of Bambara groundnut samples

Sample	Treatments		Raffinose		Stachyose	
	Methods	Time	Concentration (g/100g)	Loss (%)	Concentration (g/100g)	Loss (%)
BGC	Raw seeds		2.05±0.02^{a}	-	1.48±0.04^{a}	-
	Soaking	12 h	1.52±0.01^{b}	26	1.10±0.05^{b}	26
		24 h	1.28±0.07^{d}	38	0.92±0.12^{c}	38
		48 h	1.10±0.02^{e}	46	0.65±0.08^{e}	56
	Boiling	30 min	1.34±0.04^{c}	35	0.86±0.02^{d}	42
		60 min	1.17±0.03^{f}	43	0.61±0.16^{e}	59
BGW	Raw seeds		1.30±0.07^{a}	-	0.86±0.11^{a}	-
	Soaking	12 h	0.98±0.01^{b}	25	0.70±0.16^{b}	13
		24 h	0.81±0.03^{c}	38	0.62±0.05^{c}	23
		48 h	0.69±0.03^{d}	47	0.52±0.08^{d}	35
	Boiling	30 min	0.81±0.12^{c}	38	0.70±0.15^{b}	13
		60 min	0.65±0.05^{e}	50	0.60±0.07^{c}	25
BGB	Raw seeds		1.79±0.03^{a}	-	1.22±0.15^{b}	-
	Soaking	12 h	1.30±0.06^{b}	27	0.70±0.04^{b}	43
		24 h	0.89±0.01^{d}	50	0.70±0.01^{b}	43
		48 h	0.63±0.04^{f}	65	0.52±0.21^{c}	57
	Boiling	30 min	0.94±0.03^{c}	47	0.70±0.08^{b}	43
		60 min	0.74±0.01^{e}	59	0.52±0.03^{c}	57

Values are means ± standard deviation of three determinations. Means in a column not sharing a common superscript letter are significantly (P<0.05) different as assessed by LSD test. Bambara groundnut white (BGW); Bambara groundnut brown (BGB); Bambara groundnut commercial (BGC)

Soaking significantly (P<0.05) reduced the levels of the raffinose and stachyose in the samples with greater reduction observed as soaking time increased. After 48 h of soaking, the loss in raffinose and stachyose of BGC, BGB and BGW were 46, 65 and 47% and 56, 57 and 35%, respectively. Nnanna and Phillips (1988) had reported that the levels of raffinose and stachyose sugars in cowpea (*Vigna unguilata*) decreased with increase in time of soaking from 12 to 24 h. Similar observation was reported by Mulimani et al. (1997) that soaking of whole soybean seeds for 16 h led to a decrease of 80.3% of raffinose and 44.8% of stachyose. The varying degree of removal of raffinose and stachyose from legumes during soaking could be attributed to the differential solubility of individual sugars, their diffusion rates and leaching (Price et al., 1988; Upadhyay and Garcia, 1988).

Boiling led to significant (P<0.05) decrease in the levels of raffinose and stachyose in the samples compared to raw samples. Boiling for 60 min resulted in a significant (P<0.05) reduction of raffinose in BGC (1.17 g/100g), BGW (0.74 g/100g) and BGB (0.65 g/100g). The stachyose level of BGC was also reduced to 0.61 while BGW had the lowest value of 0.52 g/100g. Somiari and Balogh (1993) reported that cooking of cowpea for 50 min reduced the raffinose content to 44% and stachyose to 28.6%. Mulimani and Devendra (1998) reported a decrease in the levels of the raffinose family of sugars after cooking of soybean for 60 min. The decrease in the level of raffinose, stachyose and verbascose during cooking might be attributed to hydrolysis of these oligosaccharides into disaccharides and monosaccharides or to the formation of other compounds which are subsequently leached out (Mulimani and Devendra, 1998).

Table 4 Effect of soaking and boiling on *in-vitro* protein digestibility of Bambara groundnut samples

Treatments	Sample	In-vitro digestibility (%)
Raw seeds	BGC	56.77±0.04^{i}
	BGW	64.42±0.13^{h}
	BGB	68.35±0.09^{f}
Soaking	BGC	66.42±0.02^{g}
	BGW	73.56±0.07^{e}
	BGB	79.10±0.13^{d}
Boiling	BGC	82.89±0.01^{c}
	BGW	87.48±0.05^{b}
	BGB	89.34±0.04^{a}

Values are means ± standard deviation of three determinations. Means in a column not sharing a common superscript letter are significantly (P<0.05) different as assessed by LSD test. Bambara groundnut white (BGW); Bambara groundnut brown (BGB); Bambara groundnut commercial (BGC)

Effect of Processing Treatments on IVPD of BGC, BGB and BGW

The effects of soaking for 48 h and boiling for 60 min on IVPD of BGC, BGW and BGB are shown in Table 4. Lower IVPD level was observed in raw BGC (56.77%) compared with that of raw BGW (64.42%) and BGB (68.35%). Soaking of the samples for 48 h significantly (P<0.05) increased the IVPD of BGC, BGW and BGB to 66.42, 73.56 and 79.10%. However, highest IVPD value was observed in BGB (89.34%) compared with that of BGW (87.48%) and BGC (82.89%). The improvement in the protein digestibility of all the samples after soaking may be due to leaching-out of phytic acid, tannin and polyphenols which is known to interact with protein to form complexes. Heat processing has been reported to increase the digestibility of protein by destroying protease inhibition (Abbey and Benezi, 1988). Contrary to our findings, Osman (2007) and Yagoub and Abdalla (2007) found that cooking significantly decreased IVPD in Dicholas lablab seeds and bambara groundnut. Improvement of protein digestibility after boiling could be attributed to the reduction or elimination of different antinutrients. Thus, phytic acid, as well as condensed tannins and polyphenols are known to interact with protein to form complexes. These interactions could increase the degree of cross-linking, decreasing the solubility of proteins making protein complexes which impair protease access to labile peptide bonds (Genovese and Lajolo, 1996). In addition, thermal processing promoted structural changes of protein such as globulin, thereby increasing chain flexibility and accessibility to proteases (Swaisgood and Catignani, 1991).

Conclusion

Soaking was more effective in reducing the antinutritional factors of the samples with the control seeds exhibiting lower values than the newly developed seeds. Processing methods reduced the oligosaccharides content of the samples. The raffinose content of BGB and BGW were significantly lowered after boiling for 60 min than that of BGC. However, BGC had lower stachyose content compared with BGB and BGW after soaking for 48 h. Soaking and boiling led to an improvement in protein digestibility of the seeds with BGB having the highest values after boiling for 60 min.

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