***Original Research Article***

**CHARACTERISATION OF PRETREATED AFRICAN YAM BEAN AND BAMBARA GROUNDNUT SEED COATS FOR POSSIBLE USE IN FOOD FORMULATIONS**

**ABSTRACT**

The study focused on characterising pretreated African yam bean (AYB) and Bambara groundnut (BGN) seed coats for possible use in food formulations. The AYB and BG seed coats were dehulled to separate the seed coats from the cotyledon. The seed coats were pretreated using warm water, sodium bicarbonate solution, wild fermentation. The untreated served as control. The pre-treated seed coats were dried and milled to obtain their flours. The different flour samples were subjected to phytochemical, antinutrient, antioxidant and dietary fibre analyses using standard methods. The phytochemical result of the BG seed coat showed that phenol content ranged from 3.58 to 7.04 mgGAE/g and flavonoid ranged from 0.04 to 0.15 mgRUT/g, respectively. The phenol and flavonoid contents of AYB ranged from 3.68 to 17.07 mgGAE/g and 0.06 to 0.43 mgRUT/g, respectively. The antioxidant activities of the seed coats were obtained as FRAP (4.01-7.48; 5.88-23.70 mg/g), DPPH (21.16-86.35; 13.13-90.43%) and ABTS (0.01-0.03; 0.02 to 0.03 Mmol/g) for BG and AYB seed coats, respectively. The dietary fibre results showed that the BG seed coat had insoluble and soluble fibres of 26.70-49.91 and 17.40-18.28% when compared to 38.23-59.13 and 14.44-16.26% for the AYB seed coat, respectively. Besides, the phytate (4.53-6.18 mg/100g), oxalate (0.09-0.23 mg/g) and trypsin inhibitor (13.87-20.79%) contents of BG were significantly (p<0.05) similar to 2.06-6.59 mg/100g; 0.18-0.41 mg/100g and 18.96-27.45% obtained for AYB, respectively. The findings concluded that pretreatment methods had varying effects on the phytonutrients and anti-nutrient compositions of AYB and BG seed coats and that the seed coats of these legumes could be an essential raw material in functional food formulations.

*Keywords:* Seed coats; Pretreatment; African yam bean; Bambara groundnut; phytochemicals; antinutrients; dietary fibre

1. **INTRODUCTION**

Legume seed coats are a rich source of dietary fibre, making them a promising alternative for food formulations. However, these seedcoats also contain significant antinutritional factors (ANFs), which can negatively impact human health if not properly managed. ANFs are naturally occurring compounds that can bind to nutrients, making them less accessible for absorption in the body. Common ANFs in legume seedcoats include trypsin inhibitors, phenolic compounds, phytates, cyanogenic compounds, lectins, and saponins [1].

Various pretreatment methods have been developed to utilise legume seed coats as a dietary fibre source while minimising the impact of ANFs. These methods aim to reduce the levels of ANFs to sublethal levels, making the seedcoats safe for human consumption. These pretreatment methods include fermentation, soaking in sodium bicarbonate and warm water [2, 1]. These pretreatment methods can be combined individually to achieve optimal results. By reducing the levels of ANFs in legume seed coats, these methods can help unlock the nutritional potential of these seedcoats and make them a valuable source of dietary fibre in food formulations.

This study therefore, focused on pretreating underutilised legume seed coats of Bambara groundnut and African yam bean to enhance their food potential in various food formulations. This would contribute to increased utilisation of these underutilised legumes, which hitherto has been limited due to their long cooking times and the presence of antinutrients in their seeds, thereby contributing significantly to food and nutrition security in the regions where they are being cultivated.

1. **MATERIALS AND METHODS**

**2.1 Materials**

African yam bean (*Sphenostylis stenocarpa*) and Bambara groundnut (Vigna subterranea L*.* *Verdc*) seeds were procured from Ago Aduloju market in Ado Ekiti, Ekiti State, Nigeria. All other reagents and chemicals used were of analytical grades.

**2.2 Experiment Design**

Factorial design (26, 2 legumes and 6 pretreatments) was used for the characterisation of the pre-treated Bambara groundnut and African yam bean seed coats.

**2.3 Dehulling and Pretreatment of African Yam Bean and Bambara groundnut Seed Coats**

The seeds were sorted manually to remove extraneous materials and diseased seeds. The selected seeds were then soaked for 12 h in cold water at room temperature (28±2 oC) and dehulled manually [3]. The seed coats of each legume were divided into four (4) portions and pre-treated. The first portion (sample A) was soaked in warm water for 3 and 4 h at 55 oC, and the second portion (sample B) was soaked in sodium bicarbonate solution at different concentrations of 2 and 3% for 1 h at ambient temperature (28 ±2 oC). The third portion (sample C) was wild fermented for 24 and 48 h at room temperature (28 ±2 oC). The fourth portion (sample D), which served as the control, consisted of untreated seed coats. The pretreated seed coats were dried at 55 oC in a hot air oven (MFRS Unicorn Instruments, India) to constant

African yam bean seed

 Sorting

Soaking (in cold water for 12 h, 28 oC)

Dehulling

Pretreatments (using warm water, sodium bicarbonate

 solution and fermentation)

Drying (55 oC, 3 h)

Milling

Sieving (<100 µm)

Pretreated African yam bean

seed coat flour

Fig. 1. Flow chart for the production of pretreated African yam bean seed coat flour

Bambara groundnut seed

 Sorting

Soaking (in cold water for 12 h, 28 oC)

Dehulling

Pretreatments (using warm water, sodium bicarbonate

 solution and fermentation)

Drying (55 oC, 3 h)

Milling

Sieving (<100 µm)

Pretreated Bambara groundnut seed

seed coat flour

Fig. 2. Flow chart for the production of pretreated Bambara groundnut seed coat flour

weight. The dried seed coats were milled in a grinder (Mixer grinder. MX-AC 210S, Panasonic, JAPAN), sieved (< 100 µm) and stored for further analysis. The pretreatment process is shown in Figures 1 and 2.

 **2.4 Determination of Total Phenol**

Total phenol was determined according to the method of [4]. Sample (0.2 g) of the seed coats flour was mixed with 2.5 ml of 10% Folin-Ciocalteau’s reagent and 2 ml of 7.5% sodium carbonate. The reaction mixture was incubated for 40 min at 45 o C, and the absorbance was measured at 700 nm in the UV-visible spectrophotometer (JENWAY 6405 Model, UK and England).

**2.5 Determination of Flavonoid**

Flavonoid was determined according to the method of [5]. Approximately 0.25 ml of the sample was dissolved in distilled water, 75 μl of 5% NaNO2 solution, 0.150 ml of freshly prepared 10% AlCl3 solution and 0.5 ml of 1 M NaOH solution was added. The mixture was allowed to stand for 5 min, and the absorption was measured at 510 nm while the final result was expressed as quercetin equivalents.

**2.6 FRAP Determination**

Ferric reducing antioxidant power (FRAP) of the sample was determined according to the [6] method; 0.25 ml of the sample was mixed with 0.25 ml of 200 mM of Sodium phosphate buffer pH 6.6 and 0.25 ml of 1% KFC. The mixture was incubated at 50 o C for 20 min; after that, 0.25 ml of 10% TCA was also added and centrifuged at 2000 rpm for 10 min, 1ml of the supernatant was mixed with 1ml of distilled water and 0.1% of FeCl3 and the absorbance was measure at 700 nm.

**2.7 DPPH Determination**

DPPH radical scavenging activity of the sample was determined as described by [6]. A known volume of sample extract or reference compound, ascorbic acid, was added to a methanolic solution of DPPH (0.03 mM). Both solutions were kept in a dark chamber for 30 min before measuring the absorbance at 517 nm. Free radical scavenging ability was calculated as a percentage of DPPH discolouration as follows:

ABTS radical scavenging activity (%) = 100 - $\left[\frac{A\_{c}}{A\_{s}}\right] x 100$

Where As = absorbance of the standard and Ao = sample absorbance.

**2.8 ABTS Determination**

ABTS radical scavenging activity and total antioxidant activity were determined by the ABTS test described by [6]. 2,2’-azinobis (3-ethylbenzothiazoline-6- sulfonic acid) diammonium salt (ABTS.+ ) decolourisation The procedure involved pregeneration of ABTS.+ radical cation by mixing 7 mM ABTS stock solution with 2.45 mM potassium persulfate and incubated for 12–16 h in the dark at room temperature until the reaction was completed and the absorbance was stable—the absorbance of the ABTS. + solution was equilibrated to 0.70 (± 0.02) by diluting with water at room temperature—the predetermined volume of ABTS. + solution was mixed with the known volume of the test sample. The absorbance was measured at 734 nm after 6 min. The percentage inhibition of absorbance was calculated and plotted as a function of the concentration of standard and sample to determine the trolox equivalent antioxidant concentration (TEAC).

ABTS radical scavenging activity (%) = 100- [Ac/As] ×100

Where = Ac – absorbance of sample, As- absorbance of control

**2.9 Determination of the Dietary Fibre**

Insolube and soluble fibre were determined using the AOAC [7]. A gelatinised, dry, defatted food sample was enzymatically digested with alpha-amylase, amyloglucosidase and protease to break down the starch and protein components. The total fibre content of the sample was determined by adding 95 % ethanol to the solution to precipitate the fibre. The solution was filtered, and the fibre was collected, dried and weighed.

**2.10 Determination Trypsin Inhibitor**

The trypsin inhibitory activity was determined according to the method described by [8]. The trypsin inhibitory activity was assessed by estimating the difference between the enzyme activity in the presence (sample) and the absence of inhibitors (standard). The sample's optical density (OD) values were subtracted from the standard OD value and plotted on the graph against the volume of crude extract. The volume of extract, corresponding to half of the standard OD value, was considered as the volume of the sample, giving 50% inhibition. This is defined as one Trypsin Inhibitory Unit (TIU) [9]. The following formulae estimated the TIU and specific activity [10].

Specific activity (TIU per mg protein) = $\frac{TIU per gram of seed}{ing of protein}$

**2.11 Determination of Oxalate**

Oxalate was determined according to the method described by [11]. 2 g of the sample was digested with 10 ml 6 M HCl for one hour and made up to 250 ml in a volumetric flask. The pH of the filtrate was adjusted with conc. NH4OH solution until the colour of the solution changed from salmon pink to a faint yellow colour. After that, the filtrate was treated with 10 ml of 5% CaCl2 solution to precipitate the insoluble oxalate. The suspension was centrifuged at 2500 rpm, after which the supernatant was decanted and precipitated wholly dissolved in 10 ml of 20% (v/v) H2SO4. The total filtrate resulting from the dissolution in H2SO4 is made up to 300 ml. An aliquot of 125 ml of the filtrate was heated until near boiling point and then titrated against 0.05 M of standardised KMnO4 solution to a faint pink colour, which persisted for about 30 s after which the burette reading was taken. The oxalate content was evaluated from the titre value. The overall redox reaction is:

$ $Oxalate Equation:

$2M\_{n}O\_{4}^{-}+5C\_{2}O\_{4}^{2-}+16H^{+} \rightarrow 2Mn^{2+}+H\_{2}O+10CO\_{2}$

**2.12 Determination of Phytate**

Phytate was determined according to the method of [12]. Sample 4 g was soaked in 100 ml of 2% HCl for 2 h and filtered through Whatman No. 2 filter paper. After which, 25 ml of the filtrate was placed in a conical flask, and 5 ml of 0.3% ammonium thiocyanate solution was added, after which 53.5% of distilled water was added, and this was titrated against a standard iron (III) chloride solution until a brownish yellow colour persisted for 5 min. The phytate content was expressed as the percentage (%) of the sample.

**2.13 Determination of Tannin**

Tannin content was determined according to the method described by [13]. About 2 g of the sample was weighed into a sample bottle, and 10 ml of 70 % aqueous acetone was added and adequately covered. The bottle was put in an ice bath shaker and shaken for 2 hours at 30 o C. The solution was centrifuged, and the supernatant was stored in ice. Approximately 0.2 ml was pipetted into a test tube and added 0.8 ml of distilled water. A standard tannic acid solution was prepared from 0.5 mg/ml of the stock, and the solution was made up of 1 ml of distilled water. 0.5 ml of Folin Ciocalteu’s reagent will be added to the sample and standard, followed by 2.5 ml of 20% Na2CO3. The solution was vortexed and incubated for 40 min at room temperature; its absorbance was read at 725 nm. The tannin concentration in the sample was calculated from a standard tannic acid curve.

1. **RESULTS AND DISCUSSION**

**3.1 Effect of Pretreatment on Phytochemical contents of African Yam Bean and Bambara groundnut Seed Coats**

The total phenols (Figure 3a and b) of the pretreated African yam bean (AYB) and Bambara groundnut (BGN) seed coats ranged from 3.68 to 17.07 mg GAE/g and 3.58 to 7.04 mg GAE/g respectively. The total phenol result showed that the control sample had the lowest content. The low concentration of phenolic compounds in the control sample might be attributed to the low solubility of polyphenols in water at low temperatures [14, 15]. Addition of sodium bicarbonate (NaHCO3) into the soaking medium significantly (p<0.05) increased the total phenol content of AYB seed coats, with sample ASC-3% having the highest total phenol content of 17.07 mg GAE/g. This might be due to the extraction power of total polyphenols in NaHCO3 through the pH increase of the extraction solution [16].

The effect of pretreatment on the mean values of total phenol in the BG seed coats, as shown in Figure 3b, showed that the phenol content ranged from 3.58 to 7.04 mg GAE/g. The sodium bicarbonate- and warm water-pretreated samples had the significantly (p<0.05) highest (7.04 mgGAE/g) and lowest (3.58 mgGAE/g) phenol values, respectively. The low value recorded in the warm water pretreatment suggested that thermal degradation of phenol compounds might have occurred, as previously observed [17]. The current result is in consonant with 3.60-11.00 mgGAE/g reported for whole and dehulled BG seeds [18].



B

A

**Fig. 3. Effect of pretreatment on the total phenol of (a) African yam bean (AYB) and (b) Bambara groundnut (BG) seed coats**

**Key:** ACW=AYB seed coat soaked in cold water; AWW-4 h = AYB seed coat soaked in warm water for 4 h; AWW-3 h=AYB seed coat soaked in warm water for 3 h; ASC-2%= AYB seed coat soaked in 2% NaCO3; ASC-3% =AYB seed coat soaked in 3% NaCO3; AF-24 h= AYB seed coat fermented for 24 h; AF-48 h= AYB seed coat fermented for 48 h; BCW=BG seed coat soaked in cold water; BWW-4 h = BG seed coat soaked in warm water for 4 h; BWW-3 h=BG seed coat soaked in warm water for 3 h; BSC-2%= BG seed coat soaked in 2% NaCO3; BSC-3% =BG seed coat soaked in 3% NaCO3; BF-24 h= BG seed coat fermented for 24 h; BF-48 h= BG seed coat fermented for 48 h

Flavonoids are a large group of ubiquitous molecules synthesised by plants [19]. Hence, the flavonoid values of the pretreated AYB seed coats, as shown in Figure 4a and b, ranged from 0.06 to 0.43 mgRUT/g and 0.04-0.15 mgRUT/g, respectively. The control sample had the lowest flavonoid content (0.06 mgRUT/g). The low flavonoid content observed in the control sample could be due to the leaching or diffusion of flavonoids into the soaking medium [20]. It can be observed that sodium bicarbonate pretreatment significantly (p<0.05) increased the flavonoid content of AYB and BG seed coat, with sample ASC-3% having the highest (0.43 mgRUT/g) and sample BSC-2% (0.15 mgRUT/g) respectively. The increased flavonoid content could be due to the effect of alkaline conditions on the extraction and release of flavonoids. Sodium bicarbonate, an alkaline compound, can alter the pH of the seed coats and create an environment that favours the extraction and solubility of flavonoids [21]. Interestingly, the increase in flavonoids in association with low NaHCO3 concentration had been previously reported in legumes like Tartary buckwheat sprouts [22, 21, 20].



A

B

**Fig. 4. Effect of pretreatment on the total flavonoids of (a) African yam bean (AYB) and (b) Bambara groundnut (BG) seed coats**

**Key:** ACW=AYB seed coat soaked in cold water; AWW-4 h = AYB seed coat soaked in warm water for 4 h; AWW-3 h=AYB seed coat soaked in warm water for 3 h; ASC-2%= AYB seed coat soaked in 2% NaCO3; ASC-3% =AYB seed coat soaked in 3% NaCO3; AF-24 h= AYB seed coat fermented for 24 h; AF-48 h= AYB seed coat fermented for 48 h; BCW=BG seed coat soaked in cold water; BWW-4 h = BG seed coat soaked in warm water for 4 h; BWW-3 h=BG seed coat soaked in warm water for 3 h; BSC-2%= BG seed coat soaked in 2% NaCO3; BSC-3% =BG seed coat soaked in 3% NaCO3; BF-24 h= BG seed coat fermented for 24 h; BF-48 h= BG seed coat fermented for 48 h

**3.2 Effect of Pretreatment on antioxidant activities of African Yam Bean and Bambara groundnut Seed Coats**

The ferric-reducing antioxidant power (FRAP) mean values for pretreated AYB and BG seed coats ranged from 5.88 to 23.70 mg/g and 2.80 to 7.48 mg/g, respectively as shown in Figures 5a and b. The control samples of AYB and BG had the lowest FRAP values (5.88 mg/g; 2.80 mg/g). The low FRAP values observed in the control samples could be because cold temperature used for the soaking before dehulling might have triggered enzymatic reactions or altered pH levels, which could have disrupted these compounds and reduced their antioxidant capacity [23]. Also, soaking in cold water may enhance the extraction of non-antioxidant components from the seed coats (24). This could dilute the concentration of the antioxidants, leading to a decrease in FRAP values.

Sodium bicarbonate pretreated sample (ASC-3%) had the highest FRAP value (23.70 mg/g) and BSC-2% (7.48 mg/g). The observed higher FRAP value could be due to structural changes in the seed coats by sodium bicarbonate, leading to improved accessibility of antioxidant compounds. This increased accessibility could have enhanced the extraction efficiency of these compounds during subsequent analyses, resulting in higher FRAP values [25]. The current observation with the sodium bi-carbonate concentration pretreatments significantly (p<0.05) increased the FRAP value of the AYB and BG seed coats samples, although contrary to the previous study that reported a decrease in FRAP of faba beans pretreated with sodium bicarbonate [26]. The FRAP assay is used to assess the bioavailability of antioxidants in foods and to investigate the effects of storage, processing, and cooking methods on the total antioxidant content of food. The FRAP assay can be employed as a quality control check device to detect adulteration of food [25].



B

A

**Fig. 5. Effect of pretreatment on the Ferric Reducing Antioxidant Power of (a) African yam bean and (b) Bambara groundnut seed coats**

**Key:** ACW=AYB seed coat soaked in cold water; AWW-4 h = AYB seed coat soaked in warm water for 4 h; AWW-3 h=AYB seed coat soaked in warm water for 3 h; ASC-2%= AYB seed coat soaked in 2% NaCO3; ASC-3% =AYB seed coat soaked in 3% NaCO3; AF-24 h= AYB seed coat fermented for 24 h; AF-48 h= AYB seed coat fermented for 48 h; BCW=BG seed coat soaked in cold water; BWW-4 h = BG seed coat soaked in warm water for 4 h; BWW-3 h=BG seed coat soaked in warm water for 3 h; BSC-2%= BG seed coat soaked in 2% NaCO3; BSC-3% =BG seed coat soaked in 3% NaCO3; BF-24 h= BG seed coat fermented for 24 h; BF-48 h= BG seed coat fermented for 48 h

The Diphenylpicrylhydrazyl (DPPH) is a rapid, simple, inexpensive and widely used method to measure the ability of compounds to act as free radical scavengers or hydrogen donors and to evaluate the antioxidant activity of foods [27]. The DPPH results (Figures 6a and b) for AYB and BG seed coats ranged from 13.13 to 90.43% and 21.16 to 86.35%, respectively. The results in Figure 4 show that the control sample had the highest DPPH value (90.43%). The higher DPPH value observed on the control sample could be due to the breaking down of the cell walls of the seed coats by water molecules, thereby releasing the bioactive compounds (DPPH) and making them more accessible for extraction [28, 29].

The AYB seed coat sample fermented for 24 h (AF-24 h) had the lowest DPPH (13.13%). Notably, many lactic acid bacteria involved in fermentation possess enzymatic and non-enzymatic antioxidative mechanisms, which could cause a reduction in the antioxidant capacity of food materials [30]. Also, the DPPH reduction could be due to the removal of soluble compounds during fermentation, which got discarded with the soaking solution. The current result followed similar observations on pretreated faba beans [31].

Using sodium bi-carbonate in BG seed coat pretreatment reduced the DPPH value (90.43 to 21.16 %). The reduced DPPH value observed in the Bambara groundnut seed coat sample pretreated with sodium bi-carbonate could be attributed to its antioxidant properties. Sodium bicarbonate, or baking soda, is an essential compound that can help neutralise free radicals and oxidative stress [32]. The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is commonly used to measure the antioxidant capacity of compounds. The DPPH radical is scavenged by antioxidants, leading to a decrease in its absorbance, indicating a reduction in free radicals and oxidative stress [32]. Therefore, using sodium bicarbonate in seed coat pretreatment likely enhanced antioxidant activity and reduced levels of free radicals, as indicated by the lower DPPH value. The DPPH range (21.16 to 86.35%) observed in this study is more significant than 38.90 to 57.10% reported for Bambara groundnut seeds [33].



B

A

**Fig. 6. Effect of pretreatment on the diphenylpicrylhydrazyl (DPPH) of (a) African yam bean and (b) Bambara groundnut seed coats**

**Key:** ACW=AYB seed coat soaked in cold water; AWW-4 h = AYB seed coat soaked in warm water for 4 h; AWW-3 h=AYB seed coat soaked in warm water for 3 h; ASC-2%= AYB seed coat soaked in 2% NaCO3; ASC-3% =AYB seed coat soaked in 3% NaCO3; AF-24 h= AYB seed coat fermented for 24 h; AF-48 h= AYB seed coat fermented for 48 h; BCW=BG seed coat soaked in cold water; BWW-4 h = BG seed coat soaked in warm water for 4 h; BWW-3 h=BG seed coat soaked in warm water for 3 h; BSC-2%= BG seed coat soaked in 2% NaCO3; BSC-3% =BG seed coat soaked in 3% NaCO3; BF-24 h= BG seed coat fermented for 24 h; BF-48 h= BG seed coat fermented for 48 h

ABTS in seed coats is attributed to high isoflavone content [34]. The results presented in Figures 7a and b showed that the 2, 2’ –azino-bis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS) of pretreated AYB and BG seed coats ranged from 0.02 to 0.03 Mmol/g and 0.01 to 0.03 Mmol/g respectively. The African yam bean seed coat was observed to have higher ABTS values than the Bambara groundnut seed coat. For the African yam bean seed coat, the control sample (ACW) had the highest ABTS value (0.03 Mmol/g), which was not significantly different (p>0.05) from 0.03 Mmol/g reported for the 24 h fermented seed coat sample. The rest of the pretreatment methods (Sodium bicarbonate and warm water) significantly reduced the ABTS values. The reduction of ABTS values by Sodium bicarbonate and warm water pretreatment methods could be due to the activation of various enzymes present in the seed coat, including those involved in antioxidant metabolism and degradation processes such as **Superoxide Dismutase (SOD), Ascorbate Peroxidase (APX), Glutathione Reductase (GR) and Catalase (CAT) [35].** The presence of sodium bicarbonate might further modulate these enzymatic activities, leading to a reduction in ABTS content.

However, warm water and fermentation pretreated samples for the BG seed coat were statistically similar and had the highest ABTS values of 0.03 Mmol/g and 0.03 Mmol/g, respectively (Figure 7b). Significant differences (p<0.05) were observed in the ABTS values of the different samples. The result showed that using the sodium bi-carbonate pretreatment method had no significant effect (p>0.0) on the ABTS content of pretreated BG seed coats. However, warm water pretreatment and fermentation methods were observed to have significantly (p<0.05) increased the ABTS values of the samples. The increase in ABTS may be due to the role of hydrolytic enzymes that released/mobilised bound polyphenolic compounds due to the release of a bound form of phytochemicals present and high total phenol content modulated during fermentation [4]. Values obtained in this study are lower than the average value reported by [36] to be 0.28 Mmol/g.



B

A

**Fig. 7. Effect of pretreatment on the 2, 2’ – casino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) of (a) African yam bean and (b) Bambara groundnut seed coats**

**Key:** ACW=AYB seed coat soaked in cold water; AWW-4 h = AYB seed coat soaked in warm water for 4 h; AWW-3 h=AYB seed coat soaked in warm water for 3 h; ASC-2%= AYB seed coat soaked in 2% NaCO3; ASC-3% =AYB seed coat soaked in 3% NaCO3; AF-24 h= AYB seed coat fermented for 24 h; AF-48 h= AYB seed coat fermented for 48 h; BCW=BG seed coat soaked in cold water; BWW-4 h = BG seed coat soaked in warm water for 4 h; BWW-3 h=BG seed coat soaked in warm water for 3 h; BSC-2%= BG seed coat soaked in 2% NaCO3; BSC-3% =BG seed coat soaked in 3% NaCO3; BF-24 h= BG seed coat fermented for 24 h; BF-48 h= BG seed coat fermented for 48 h

**3.3 Effect of Pretreatment on the Fiber Contents of African Yam Bean and Bambara Groundnut Seed Coat**

The insoluble fibre values for pretreated AYB and BG seed coats ranged from 38.23 to 59.13% and 26.70 to 49.91%, as shown in Figure 8a and b, respectively. Warm water pretreated sample had the highest insoluble fibre for African yam bean and Bambara groundnut seed coats. The high insoluble fibre observed in the warm water pretreated samples could be due to the hydration of the cell walls of the seed coats, thereby making the cell wall components more accessible for extraction, which subsequently increased the solubility of cellulose and hemicellulose thereby leading to a higher content of insoluble fibre. By implication, warm water pretreatment is essential in enhancing the insoluble fibre content of the Bambara seed coat, which can help reduce colon cancer when consumed in Bambara seed coat products [37]. Fermented pretreated seed coats had the lowest insoluble fibre content for both legume seed coats. During fermentation, microorganisms produce enzymes that break down complex carbohydrates, including insoluble fibres [38]. This could have led to the low insoluble fibre observed on the fermented pretreated sample. A high proportion of Insoluble dietary fibre observed in the warm water pretreated samples is advantageous because of its potential application as a functional ingredient in confectionery or in the preparation of low-fat, high-fibre dietetic products [39]. The insoluble fibre values observed in this study are higher than the 10.14% reported for sesame seed coat [40]. The highest value of insoluble dietary fibre for Bambara groundnut (49.9%) is higher than the value reported by [41] who reported 17.1% for soybeans. Legumes and their seed coat are high in dietary fibre, complex carbohydrates with low glycemic index, and bioactive compounds but low in saturated fat and no cholesterol [42]. These dietary components can promote health and longevity by increasing insulin production and preventing chronic diseases such as diabetes, cancer, cardiovascular disease and obesity [43].



B

A

**Fig. 8. Effect of pretreatment on the insoluble fibre of (a) African yam bean (AYB) and (b) Bambara groundnut (BG) seed coats**

**Key:** ACW=AYB seed coat soaked in cold water; AWW-4 h = AYB seed coat soaked in warm water for 4 h; AWW-3 h=AYB seed coat soaked in warm water for 3 h; ASC-2%= AYB seed coat soaked in 2% NaCO3; ASC-3% =AYB seed coat soaked in 3% NaCO3; AF-24 h= AYB seed coat fermented for 24 h; AF-48 h= AYB seed coat fermented for 48 h; BCW=BG seed coat soaked in cold water; BWW-4 h = BG seed coat soaked in warm water for 4 h; BWW-3 h=BG seed coat soaked in warm water for 3 h; BSC-2%= BG seed coat soaked in 2% NaCO3; BSC-3% =BG seed coat soaked in 3% NaCO3; BF-24 h= BG seed coat fermented for 24 h; BF-48 h= BG seed coat fermented for 48 h

Figures 9a and b present the soluble fibre contents of pretreated AYB and BG seed coats. The soluble fibre contents ranged from 14.44 to 16.26% for the pretreated African yam bean seed coat and 17.40 to 18.28% for the pretreated Bambara groundnut seed coat. The result shows that the Bambara groundnut seed coat had higher soluble fibre when compared to the African yam bean seed coat, suggesting that the Bambara groundnut seed coat could be a good source of soluble fibre. The result further showed that sodium bi-carbonate and fermentation pretreatment methods significantly (p<0.05) reduced the soluble fibre content of AYB seed coats with increased time. The low soluble dietary fibre could be linked to the rapid action of the pretreatments accompanied by a breakdown of short-chain fatty acids through higher consumption by the probiotics in the seed coat [44]. Warm water pretreatment for 4 h (AWW-4h) increased the soluble fibre content of both African yam bean and Bambara groundnut seed coats. The increased soluble dietary fibre observed in sample AWW-4h could be an advantage since soluble dietary fibre has been reported to help lower blood cholesterol and glucose levels [45, 46]. The dietary fibre (Insoluble and Soluble) reported in this study is higher than the 2.3% previously reported for cotyledon of African yam bean [47]. Reports showed that soluble fib*re* attracts water and turns to gel during digestion, slowing food digestion and increasing satiety [48]. This suggests that the warm water pretreatment process could be a better process for enhancing the Bambara seed coat's soluble and soluble fibre contents.

B

A



**Fig. 9. Effect of pretreatment on the soluble fibre of (a) African yam bean (AYB) and (b) Bambara groundnut (BG) seed coats**

**Key:** ACW=AYB seed coat soaked in cold water; AWW-4 h = AYB seed coat soaked in warm water for 4 h; AWW-3 h=AYB seed coat soaked in warm water for 3 h; ASC-2%= AYB seed coat soaked in 2% NaCO3; ASC-3% =AYB seed coat soaked in 3% NaCO3; AF-24 h= AYB seed coat fermented for 24 h; AF-48 h= AYB seed coat fermented for 48 h; BCW=BG seed coat soaked in cold water; BWW-4 h = BG seed coat soaked in warm water for 4 h; BWW-3 h=BG seed coat soaked in warm water for 3 h; BSC-2%= BG seed coat soaked in 2% NaCO3; BSC-3% =BG seed coat soaked in 3% NaCO3; BF-24 h= BG seed coat fermented for 24 h; BF-48 h= BG seed coat fermented for 48 h

**3.4 Effect of Pretreatment on the Antinutritional Content of African Yam Bean and Bambara Groundnut Seed Coats**

Knowing phytate levels in foods is necessary because a high concentration of phytate could adversely affect the digestibility of foods [49]. The phytate contents of pretreated AYB and BG seed coats in Table 1 ranged from 2.06 to 6.59 mg/100g and 4.53 to 6.59 mg/100g, respectively. The result showed no significant difference (p>0.05) in the phytate content of the pretreated samples with that of the control sample except for sample SC-2%, which was statistically found to have the lowest phytate content. Sodium bicarbonate is an alkaline compound that can increase the pH of a solution when added [50]. Phytate, phytic acid, is more stable and less soluble under acidic conditions. By raising the pH, sodium bicarbonate creates a more favourable environment for the breakdown of phytate [51]. This could have led to the decreased phytate content in the sodium bicarbonate pretreated sample. The phytate contents recorded in the present study are found to be lower than 11.12 mg/100g reported for lima bean seed coat [49] but agree with the 2 to 9 mg/100g safe range reported by [52]. Phytic acid binds to phosphorus and converts it to phytate, an indigestible substance, thereby decreasing the bioavailability of this element for absorption [52].

Oxalates from plant sources have been known to cause irreversible oxalate nephrosis when ingested in large doses [53]. Oxalate nephrosis is an acute or chronic decrease in kidney function associated with the deposition of calcium oxalate crystals in kidney tubules [54]. As presented in Table 1, oxalate values for pretreated AYB and BG seed coats ranged from 0.18 to 0.41 mg/100g and 0.09 to 0.23 mg/100g, respectively. From the result, it was observed that fermentation for 48 h and the use of 3% sodium bicarbonate increased the oxalate contents of the legume seed coats. However, warm water pretreatment reduced the oxalate content of the legume seed coats. The low oxalate content observed in the warm water pretreated samples of the legume seed coats could be because oxalates are generally more soluble in warm or hot water than cold water [55]. The moderate temperature of the water used during pretreatment may have helped dissolve and extract oxalate from the legume seed coats, reducing their content [54]. High oxalate meals contain more than 50 mg per serving, while low oxalate content meals have less than 2 mg per serving [56]. Given those above, the oxalate level of all the samples poses no danger in diet, as [52] reported a safe, normal range of 2 – 9 mg/100g for oxalate, implying that the use of these seed coats in food formulation will not pose any health threat to the consumer. The oxalate values recorded in this study are lower than the average safe level (2 to 9 mg/100g) reported by [52], implying that the use of Bambara groundnut seed coat in food formulations will not pose any health threat to the consumer.

Trypsin inhibitor is found in all legumes in varying degrees and inhibits proteolytic enzyme functions, especially trypsin and chymotrypsin in humans, reducing protein digestibility and nutritive value [57]. The trypsin inhibitor activity (TIA) of the pretreated AYB and BG seed coats (Table 1) ranged from 18.96 to 29.42% and 13.87 to 20.79 %, respectively. Samples SC-3% had the highest trypsin inhibitor for AYB and BG seed coats. The high trypsin inhibitor observed in the sodium bicarbonate pretreated seed coat samples could be due to the triggering of enzymatic reactions by sodium bicarbonate, which may have caused a breakdown of the cell wall components, releasing more trypsin inhibitors. This may have increased the solubility and bioavailability of these inhibitors, leading to a higher content in the seed coats [58]. Also, sodium bicarbonate pretreatment may have enhanced the antioxidant activity of the seed coats, which could have contributed to the increased trypsin inhibitor content. This is because antioxidants can protect the seed coats from oxidative damage, allowing more trypsin inhibitors to be preserved and measured [59]. The warm water pretreated sample (WW-3 h) had the lowest trypsin inhibitor for both legume seed coats. This reduction in trypsin inhibitor content by the warm water pretreatment method may be due to the leaching and thermal effects. A previous study [60] had earlier reported that heat application methods such as boiling, microwaving and other heat application processes could reduce the trypsin inhibitor content of legumes, which agrees with the observation made in the present study.

**Table 1. Effect of pretreatment on the anti-nutritional content of African yam bean and Bambara groundnut seed coats**

|  |  |  |  |
| --- | --- | --- | --- |
| **Sample/Pretreatment** | **Phytate****(mg/100g)** | **Oxalate****(mg/100g)** | **Trypsin inhibitor activity****(%)** |
| **AYB** | **BG** | **AYB** | **BG** | **AYB** | **BG** |
| CW | 4.94ab±0.00 | 4.53a±0.58 | 0.32bc±0.06 | 0 0.18b±0.00 | 23.53c±0.38 | 18.55bc±0.84 |
| WW-3 h | 6.59a±1.17 | 4.94ab±0.00 | 0.18c±0.00 | 0.09c±0.00 | 18.96def±1.41 | 13.87d±0.50 |
| WW-4h | 3.71ab±0.58 | 4.94ab±0.00 | 0.27cd±0.00 | 0.09c±0.00 | 24.61c±0.76 | 18.77bc±0.23 |
| SC-2% | 2.06b±0.58 | 4.53ab±0.58 | 0.18c±0.00 | 0.09c±0.00 | 24.99c±1.53 | 17.50c±1.19 |
| SC-3% | 4.54ab±1.24 | 6.59a±0.00 | 0.36ab±0.00 | 0.23a±0.06 | 29.42a±0.31 | 20.79a±1.49 |
| F-24 h | 5.77a±0.00 | 4.53ab±0.58 | 0.27cd±0.00 | 0.18b±0.00 | 19.01def±0.50 | 17.55c±0.80 |
| F-48 h | 6.59a±0.00 | 6.18a±0.58 | 0.41a±0.06 | 0.18b±0.00 | 27.45b±0.04 | 20.31ab±0.19 |

Values with different superscripts along a column are significantly different at p<0.05.

**Key:** ACW=AYB seed coat soaked in cold water; AWW-4 h = AYB seed coat soaked in warm water for 4 h; AWW-3 h=AYB seed coat soaked in warm water for 3 h; ASC-2%= AYB seed coat soaked in 2% NaCO3; ASC-3% =AYB seed coat soaked in 3% NaCO3; AF-24 h= AYB seed coat fermented for 24 h; AF-48 h= AYB seed coat fermented for 48 h; BCW=BG seed coat soaked in cold water; BWW-4 h = BG seed coat soaked in warm water for 4 h; BWW-3 h=BG seed coat soaked in warm water for 3 h; BSC-2%= BG seed coat soaked in 2% NaCO3; BSC-3% =BG seed coat soaked in 3% NaCO3; BF-24 h= BG seed coat fermented for 24 h; BF-48 h= BG seed coat fermented for 48 h

1. **CONCLUSION**

The results of this study have shown that the pretreatment methods used significantly affected the phytochemical contents, antioxidant activities, and fibre contents of African yam bean and Bambara groundnut seed coats, which, by extension, could influence their usage in food formulations. Sodium bicarbonate pretreatment generally increased antioxidant activity and reduced phytate content, indicating that sodium bicarbonate pretreatment may be beneficial for enhancing antioxidant activity while reducing the phytate content of these legumes’ seedcoats. Warm water pretreatment, on the other hand, increased the insoluble fibre content and decreased the antioxidant activity. This suggests that warm water pretreatment could be essential in processing legume seed coats for enhanced insoluble dietary fibre. Based on different pretreatment methods, African yam bean and Bambara groundnut seed coats showed variations in oxalate content and trypsin inhibitor activity. Still, all values fell within safe ranges for consumption. This also implies that pretreated African yam bean and Bambara groundnut seed coats could be find useful applications in functional food formulations.

**Disclaimer (Artificial intelligence)**

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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