

# Antioxidant, Anti-diabetic and Sensory Properties of Bread produced from flour blends of Wheat and Pretreated African yam bean and Bambara groundnut Seed coat

## Abstract

Producing bakery products with low glycemic index is expedient to replacing the consumption of foods, such as white bread, correlated with cardiovascular diseases. This research looked at the chemical, antioxidant, anti-diabetic, and sensory properties of bread made from a mix of wheat flour (WF), pretreated African yam bean (AYB) and Bambara groundnut (BGN) seed coat.

Bread samples were produced from formulated composite flour (wheat flour, pretreated African yam bean and Bambara groundnut seed coats) obtained from mixture design in response surface methodology in the ratio of WF: AYB (78.2 g: 21.8 g) and WF: BGN (74.42 g: 25.57 g). Samples were evaluated for proximate, total phenol and antioxidant activity (DPPH and ABTS), dietary fibre (Soluble, Insoluble and Total), anti-diabetic (glycemic index, alpha-amylase and alpha glucosidase inhibition) and sensory properties. Data generated were subjected to Analysis of Variance to determine level of significant difference while means were separated by Duncan multiple range at  $p < 0.05$ .

The proximate composition of the bread samples, were moisture (30.29–33.75%), crude fibre (0.58–2.68)%, protein (7.98–11.59%), ash (1.69–2.35%), fat (1.23–2.65%), and carbohydrate (48.20–55.77%). Total phenol, DPPH and ABTS ranged between 0.87 and 3.48 mgGAE/g, 3.07–18.88% and 0.001 - 0.004 Mmol/g respectively. The insoluble, soluble and total dietary fibre were 2.91–9.28% 1.34–4.47% and 4.25–12.73% respectively. The values for the glycemic index alpha-amylase inhibition and alpha-glucosidase inhibition of the bread samples were in the range of 21.85–53.54, 26.99–93.31%, and 34.21–74.44% respectively. The sensory attributes of the composite bread were between liked slightly and liked moderately but the overall acceptability was not significantly different from the control sample made with 100% wheat flour. The study indicated that pretreated underutilized seed coats could be a useful ingredient in producing functional bread with appreciable nutrient profile.

**Keywords:** - Pre-treated seed coats, African yam bean, Bambara groundnut, bread, antioxidant activity, anti-diabetic properties and sensory attributes

## 1. INTRODUCTION

Over the ages, legumes have occupied a prominent position in the supply of protein to households, especially in Nigeria, where a more significant percentage of the population lives below the poverty line and cannot afford the high cost of quality animal protein [1]. Legumes are gaining considerable interest globally because they promote healthy well-being by providing benefits beyond basic nutritional needs [2].

**Comment [UG1]:** It would be better to focus on the difference in treated and untreated sample rather than describing the range.

There is a growing global demand for legumes, particularly in Nigeria, due to heightened consumer awareness of their nutritional and health advantages. As a result, the cost of legumes has significantly risen [3]. To fulfil this requirement, it is necessary to focus on the nutritional analysis of several underutilised legumes, such as African yam bean and Bambara groundnut, to create affordable, creative, and enhanced goods. The thick seed coat of these legumes, underutilized to their full potential, makes preparation and processing challenging. Processing techniques like soaking, fermentation, and germination have been found to facilitate their processing, hence expanding their range of uses [4]. The dehulling process, known as primary processing, is a crucial step in the post-harvest handling of legumes. It plays a significant role in the processing and utilization of legumes in people's daily diets, as well as the preparation of local dishes [5]. In Nigeria, people dehull approximately 85% of legumes before consuming them. This process leads to the production of a significant amount of seed coating. In the coverings of legume seeds, you can find enzyme inhibitors like  $\alpha$ -amylase,  $\alpha$ -glucosidase, and  $\gamma$ -aminobutyric acid. These could be used as nutritional supplements [7]. Legume seed coverings also contain elements such as proteins, dietary fibre, and minerals that have beneficial effects on human health [8]. When taken in specific amounts, certain phytochemicals found in the seed coats of legumes, typically classified as anti-nutritional components, can benefit human health by preventing and treating specific diseases [9]. Regular consumption of seed coatings can effectively manage chronic diseases such as cardiovascular diseases, diabetes mellitus, hypertension, and obesity [10, 11, 12].

Consuming too much white bread can contribute to celiac disease in people sensitive to gluten, obesity, diabetes, and heart disease; hence substituting a certain percentage of wheat flour with non-wheat and fibre-rich sources can bring succor to potential consumers. Seed coats of underutilized legumes, considered as good source of dietary fibre, may serve as suitable ingredients for bread formulation [13]). Currently in Nigeria, legume seed coats are usually discarded after or during processing of legumes as waste products despite their nutritional and phytochemical potentials. Consumption of white bread is gradually increasing owing to its relative cheap price among other food items such as rice, beans and others.

Wheat flour has been reported to adversely affect consumers' health due to its high glycemic index and effects on people living with celiac disease [14, 13]. Production of commonly consumed bakery products with a low glycemic index becomes expedient because consumption of foods with a high glycemic index has been positively correlated with cardiovascular disease risk factors [7]. This study, therefore, investigated the effects of different pre-treatment methods (soaking in cold and warm water, sodium bicarbonate solution, and fermentation) on the chemical and sensory properties of bread produced from blends of wheat and pre-treated seed coats of African yam bean and Bambara groundnut.

**Comment [UG2]:** Well written introduction with clear context for the research. However, a little background on prevalence of metabolic disorders like diabetes, obesity, CVD etc and how your research could address these concerns would have been better.

## 2. MATERIALS AND METHODS

### 2.1 Materials

African yam beans (*Sphenostylisstenocarpa*) and Bambara groundnut (*Vigna subterranea* L. Verdc) were procured from the *Ago Aduloju* market in Ado Ekiti, Ekiti State. Wheat flour (Golden penny) was sourced from a recognized retailer in Ado-Ekiti, Ekiti State, Nigeria. All the reagents used were analytical grades.

### 2.2 Dehulling and Pre-treatment of Seed coat of African yam bean and Bambara groundnut

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 ( $28 \pm 2$  °C) and dehulled. Pre-treating seed coats of AYB and BGN in 2% NaHCO<sub>3</sub> yielded the best results in terms of the evaluated parameters in a previous study and this informed its choice in this present study. Dehulled seed coats were soaked in a 2% sodium bicarbonate solution for 1 h. The pre-treated seed coats were dried at 55°C in a hot air oven (MFRS Unicorn Instruments, India) to a constant weight. The dried seed coats were milled in a grinder (Mixer grinder. MX-AC 210S, Panasonic, JAPAN) to obtain flour and sieved (100 µm). The flour obtained was packaged in high-density polyethene before analyses.

Comment [UG3]: Reference is missing here.

### 2.3 Composite Flour Formulation

The flour used for bread production was obtained from blends of wheat flour, pre-treated seed coats of African yam bean, and Bambara groundnut. The formulation ratio for the composite flour was obtained from a mixture design in response surface methodology, which was evaluated from the combination of wheat flour, pre-treated African yam bean, and Bambara groundnut seed coat flour. The optimum formulation ratios were Wheat flour (WF): African yam bean (AYB) (78.2: 21.8 g) and WF: Bambara groundnut (BG) (74.42: 25.57 g). The 100% wheat flour was used as the control sample.

### 2.4 Production of Bread

All formulations were baked according to [15] method. The dough was adequately prepared by mixing the flour with weighed ingredients: 5 g salt, 40 g margarine, 20 g yeast and 60 g granulated sugar in 500 ml water, followed by stirring using a Kenwood mixer (Model A 907 D, Japan) for 5 min to obtain a dough. The dough (250 g) was allowed to undergo first proofing in a baking tin for 55 min at room temperature ( $28 \pm 2$  °C) and second proofing in a cabinet for 90 min at 30 °C, 85% RH and baked at 250 °C for 30 min. Before analyses, the bread was cooled at room temperature ( $28 \pm 2$  °C) and preserved in low-density polyethene.

### 2.5 Proximate Composition Analysis of Composite Bread

The moisture, ash, fat, crude fibre and protein content of composite bread were determined using the standard procedures of the Association of Official Analytical Chemists [16]. Moisture content was determined by drying the sample in an oven at 105°C to a constant weight. Ash was measured from the residual mass obtained after incinerating the samples at 550°C for 2 h in a muffle furnace. Fat content was determined by continuous extraction in a Soxhlet apparatus for 4 h using hexane as a solvent. After evaporation of the solvent, the fat content was obtained by the gravimetric method. Crude fibre was determined by acid and alkali digestion methods. Protein content was determined using the Kjeldahi method. Carbohydrate content was calculated by difference.

## 2.6 Total Phenol Analysis of the Composite Bread

Total Phenol was determined according to the method described by [17]. The composite bread sample (0.2 g) was mixed with 2.5 ml of 10% Folin-Ciocalteu's reagent and 2 ml of 7.5% sodium carbonate. The reaction mixture was incubated for 40 min at 45 °C, and the absorbance was measured at 700 nm in the UV-visible spectrophotometer (JENWAY 6405 Model, UK and England).

## 2.7 Antioxidant Assays of the Composite Bread

### 2.7.1 Determination of 2,2-Diphenyl-1-Picrylhydrazyl Hydrate (DPPH) radical scavenging activity of the composite bread

DPPH (2,2-diphenyl-1-picrylhydrazyl hydrate) radical scavenging activity of the composite bread samples were determined as described by [18]. The procedure involved taking a known volume of sample extract or reference compound, ascorbic acid, and adding it 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 discoloration as follows:

$$\text{DPPH radical scavenging activity (\%)} = \left[ \frac{A_s - A_o}{A_s} \right] \times 100$$

Where  $A_s$  = absorbance of the standard and  $A_o$  = absorbance of the sample.

### 2.7.2 Determination of 2,2'-azinobis (3-ethylbenzothiazoline-6- sulfonic acid (ABTS) of the composite bread

Total antioxidant activity was determined by the ABTS test described by [18]. 2,2'-azinobis (3-ethylbenzothiazoline-6- sulfonic acid) diammonium salt (ABTS.+ ) decolourization 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 ( $\pm$  0.02) by diluting with water at room temperature. The preset volume of ABTS+ solution was combined with a measured volume of the test sample. After 6 minutes, we measured the absorbance at a wavelength of 734 nm. The percentage reduction in absorbance was computed and graphed as a function of the concentration of the standard and sample in order to ascertain the trolox equivalent antioxidant concentration (TEAC). The ABTS radical scavenging activity can be calculated using the formula:

$$\text{ABTS radical scavenging activity (\%)} = 100 - \left[ \frac{A_c}{A_s} \right] \times 100$$

Where  $A_c$  represents the absorbance of the sample and  $A_s$  represents the absorbance of the control.

## 2.8 Dietary fibre of the Composite Bread

Insoluble and soluble fibre were analysed using the [16]. The food sample, which had been dehydrated and had its fat removed, was subjected to enzymatic digestion using  $\alpha$ -amylase, amyloglucosidase, and protease enzymes in order to break down the starch and protein components. The total fibre content of the sample was evaluated by precipitating all the fibre in the solution using 95% ethanol. The fluid underwent filtration, resulting in the collection, drying, and weighing of the fibre. The water-soluble and water-insoluble fibre

components were identified by filtering the sample that had undergone enzymatic digestion. The soluble fibre remained in the filtrate solution whereas the insoluble fibre was retained in the filter. The component that cannot be dissolved was gathered from the filter, dried, and measured in terms of weight. The soluble component was separated from the solution by precipitating it with 95% alcohol, then collected through filtration, dried, and measured in terms of weight. To account for any residual presence of protein and ash in the fibre, the protein and ash content of the different fractions was measured. The total fibre content can be calculated by subtracting the combined weight of protein and ash from the weight of the residue.

## 2.9 Glycemic index of the Composite Bread

The glycemic index was determined according to the method of [19]. Bread samples (50 mg) were incubated at 37 °C with 1 mg of pepsin in 10 mL HCl-KCl buffer (pH 1.5) at 40 °C for 60 min in a shaking water bath. The digest was diluted to 25 mL by adding phosphate buffer (pH 6.9) and 5 mL of  $\alpha$ -amylase solution was then added, which contained 0.005 g of  $\alpha$ -amylase in 10 mL of buffer. A 0.1-ml sample was taken from each flask every 30 min from 0 to 3 h and boiled for 15 min to inactivate the enzyme. Sodium acetate buffer (1 mL 0.4M, pH 4.75) was added, and the residual starch was digested to glucose by adding 30 mL amyloglucosidase and incubating at 60 °C for 45 min. The glucose concentration was determined by adding 200 mL of dinitrosalicylic acid colour reagent. The reaction mixtures were stopped by placing the tubes in a water bath at 100°C for 5 minutes and then cooled to room temperature. The reaction mixture was then diluted by adding 5 mL of distilled water and centrifuged (Model 90-1 LIAM MEDICAL ENGLAND) at 1200 x g. The supernatant was collected, and the absorbance was measured at 540 nm using a spectrophotometer (JENWAY 6405 Model, UK and England). The rate of starch digestion was expressed as the percentage of hydrolyzed starch per time. A 50-mg sample of glucose was used as the standard.

**Comment [UG4]:** Please recheck the reference, I could not find this information in the referred article.

## 2.10 Inhibition of alpha-amylase Activity Assay of the Composite Bread

The alpha-amylase inhibition method is used to estimate the amount of reducing sugar produced by the activity of each enzyme on buffered starch.  $\alpha$  - amylase was assayed, as reported by [20]. The substrate for the assay was 0.5 ml of 0.5% soluble starch, buffered with 0.2 ml of 0.1 M sodium acetate (pH 5.6). An aliquot of crude enzyme extract (0.3ml) was introduced into the mixture, thoroughly mixed, and then subjected to incubation at a temperature of 40°C for 30 minutes in a water bath. A volume of 1 millilitre of DNSA solution was introduced into the mixture and subjected to boiling for 5 minutes. After cooling, a 4 mL aliquot of distilled water was added before measuring the absorbance at 540 nm using a spectrophotometer. A blank solution was prepared by combining 0.3 ml of distilled water, 0.5 ml of 0.5% soluble starch, and 0.2 ml of buffer. This blank solution was then treated to the same protocols.

## 2.11 Inhibition of alpha- glucosidase Activity Assay of the Composite Bread

The inhibition of alpha-glucosidase was assessed using the method outlined by [20], utilising alpha-glucosidase derived from *Saccharomyces cerevisiae*. 50  $\mu$ l of the sample was pre-incubated with 100  $\mu$ l of  $\alpha$ -glucosidase (0.3 U/mL) for 10 min. The substrate solution, p-nitrophenylglucopyranoside (pNPG), was produced in 20 mM phosphate buffer and pH 6.9. Next, 50 microliters of a 3.0 millimolar solution of pNPG (a substrate) dissolved in a 20-millimolar phosphate buffer with a pH of 6.9 was added to initiate the reaction. The reaction mixture was subjected to incubation at a temperature of 37°C for 20 minutes, and the reaction was terminated by the addition of 2 mL of a solution containing 0.1 M Na<sub>2</sub>CO<sub>3</sub>. The alpha-glucosidase activity was assessed by quantifying the release of para nitrophenol, which has a yellow colour, from pNPG at a wavelength of 405 nm.

## 2.12 Evaluation of Sensory Attributes of the Composite Bread

The sensory features (including crust and crumb colour, taste, aroma, appearance, texture, and overall acceptability) were assessed using the methodology described in reference [21]. A panel of 75 individuals, consisting of both faculty members and undergraduate students from the Department of Food Science and Technology at Bamidele Olumilua University of Education Science and Technology in Ikere - Ekiti, was chosen. A 9-point Hedonic scale was utilised, with ratings ranging from 9 (indicating a large liking) to 1 (indicating a strong dislike).

## 2.13 Statistical Analysis

The collected data were analysed using Analysis of Variance (ANOVA) with the Statistical Analysis Software SAS (2009) for Microsoft Windows. The Duncan Multiple Range Test (DMRT) was used to separate the means, and a significance level of  $p < 0.05$  was used to determine significant differences. The analyses were performed in triplicate.

## 3. RESULTS AND DISCUSSION

### 3.1 Proximate Composition of Composite Bread

The moisture contents of the bread samples, presented in Table 1, ranged between 30.29% and 33.75%. The control sample, consisting of 100% wheat flour, exhibited the maximum moisture content at 33.75%, whereas the bread with African yam bean seed coat flour had the lowest moisture content at 30.29%. The bread samples exhibited statistically significant differences ( $p < 0.05$ ) in their moisture content. The inclusion of African yam bean and Bambara groundnut seed coat flours resulted in a significant ( $p < 0.05$ ) reduction in moisture content in the bread samples, indicating improved stability in favorable conditions. The measurement of moisture content has been utilised to assess stability and vulnerability to microbial contamination [22]. The moisture values observed in the current investigation were below the range of 30.90 to 36.00%, which was previously reported by [23] for bread made from a mixture of wheat, anchote, and soybean flours. The bread from this study has a low moisture level, which can be beneficial because high moisture can promote the growth of microorganisms, leading to faster spoilage of food products. According to the findings of [22], flour samples with lower moisture levels had better shelf stability. This is due to the fact that microbiological activities and chemical reactions, which require higher moisture levels, typically drive rotting.

The bread samples exhibited a range of crude fibre content, with values ranging from 0.58% to 4.48%. The value exhibited a statistically significant increase ( $p < 0.05$ ) in the sample when Bambara groundnut was added, with an increase from 0.58% to 4.48%. On the other hand, the addition of African yam bean seed coat only resulted in a minor rise in the content, from 0.58% to 3.88%. The control sample exhibited a very minimal value. These findings indicate that the seed coat of Bambara groundnut and African yam beans contains a significant amount of dietary fibre. The research findings of the present study align with previous reports by [24] and [25], which also highlighted the high fibre content of Bambara groundnut. High dietary fibre is beneficial for promoting regular bowel movements, reducing blood cholesterol levels, and regulating blood sugar levels [26].

The protein level of the bread samples ranged from 7.98% to 11.59%. The protein content of the various bread samples exhibited a significant difference ( $p < 0.05$ ). The protein analysis indicated that adding African yam bean and Bambara groundnut seed coat flours to wheat flour resulted in a significant ( $p < 0.05$ ) increase in the protein content of the resulting bread. The bread made with African yam bean seed coat flour exhibited the highest protein content, measuring at 11.59%. Conversely, the wheat flour-only control sample had the lowest protein content, measuring at 7.98%. The findings also indicated that the protein content of

**Comment [UG5]:** Was this difference significant? Comparatively less might be the better choice of word than a minor rise.

the African yam bean's seed coat may be higher than that of the Bambara groundnut seed coat and wheat. [27] made a similar observation about the nutritional analysis of African yam bean and Bambara nut pudding. Consuming the composite flour with enhanced protein content may potentially ameliorate protein-energy malnutrition in the malnourished group [28]. The protein values observed in this study align with a previous report by [29] (ranging from 5.38% to 12.57%) for wheat-breadfruit composite flour. In 2021, the study by [30] revealed a protein range of 12.34–14.01% for composite flour derived from wheat, acha, and African yam beans.

Comment [UG6]: ?

The bread's ash content ranged from 0.69% to 2.35%. There was a significant difference ( $p < 0.05$ ) in the ash content between the composite bread samples and the control. The findings indicated that incorporating African yam bean and Bambara groundnut seed coat flour into wheat flour led to a significant ( $p < 0.05$ ) increase in the ash content of the composite bread samples. However, it was observed that bread containing Bambara groundnut seed coat flour had a significantly ( $p < 0.05$ ) higher ash content (2.35%) compared to bread with African yam bean seed coat. The control sample had the lowest ash content. According to [31], ash residue serves as an indicator of the mineral composition of the initial food, with the mineral components typically constituting a minor proportion (less than 1%) of the food. [32] also asserted that the ash content of a sample serves as an indicator of its mineral or inorganic composition. These minerals serve as inorganic co-factors in metabolic processes. Therefore, the absence of these inorganic co-factors may lead to impaired metabolism [32]. The ash content values observed in this investigation are consistent with a previous report (1.40 to 2.82%) [33] for mixtures of African yam bean and maize seed flour.

Bread's fat content ranged from 1.23 to 2.65%. The bread containing a composite flour of Bambara groundnut seed coat had the highest fat content, measuring at 2.55%. Conversely, bread containing 100% wheat had the lowest fat content, measuring at 1.23%. A statistically significant difference ( $p < 0.05$ ) was seen in the values obtained from composite bread made with African yam bean seed coat and wheat flour, with a fat content of 1.72%, showed a statistically significant difference ( $p < 0.05$ ). Simultaneously, there was a significant difference ( $p < 0.05$ ) in the fat content between the composite bread samples made from Bambara seed coat composite flour and the control. The seed coat of legumes contains lipids, which make up approximately 1–3% of their composition [34]. The findings indicate that the seed coat of Bambara groundnut has the potential to serve as a substantial reservoir of fat. The fat content measured in the current study was consistent with the fat values of 2.62 to 5.60% reported by [25] for bread made from a mixture of wheat, yellow root cassava, and Bambara groundnut flour.

Comment [UG7]: This information is different on the table (significant difference is not indicated in the table, please clarify)

The bread's carbohydrate content ranged from 48.20% to 55.77%. The addition of African yam bean and Bambara groundnut seed coat flour resulted in a substantial decrease ( $p < 0.05$ ) in the carbohydrate content of the various bread samples. The bread containing Bambara groundnut seed coat flour had the lowest carbohydrate level, measuring at 48.20%. Conversely, the bread containing 100% wheat had the highest carbohydrate content, measuring at 55.77%. There was a statistically significant difference ( $p < 0.05$ ) between the bread made from 100% wheat flour and the bread with African yam bean seed coat (50.48%). [35] stated that a high carbohydrate level indicates that the bread would serve as a source of energy. Nevertheless, individuals aiming for weight loss may consider incorporating low-carbohydrate items into their diet. The carbohydrate content observed in this study falls within the range of 51.18 to 53.48%, which is consistent with the findings published by [25] for bread made from mixes of wheat, yellow root cassava, and Bambara groundnut flour.

Comment [UG8]: Good work

**Table 1. Proximate composition of the bread containing pre-treated African yam bean and Bambara groundnut seed coats (%).**

Sample	Moisture	Crude fibre	Protein	Ash	Fat	Carbohydrate
AYB	30.29 <sup>c</sup> ±0.57	3.88 <sup>b</sup> ±0.04	11.59 <sup>a</sup> ±0.26	2.04 <sup>b</sup> ±0.07	1.72 <sup>b</sup> ±0.19	50.48 <sup>b</sup> ±0.19
BGN	31.96 <sup>b</sup> ±0.22	4.48 <sup>a</sup> ±0.23	10.36 <sup>b</sup> ±0.04	2.35 <sup>a</sup> ±0.11	2.655 <sup>a</sup> ±0.51	48.20 <sup>c</sup> ±0.44

100% 33.75<sup>a</sup>±0.21 0.58<sup>c</sup>±0.03 7.98<sup>c</sup>±0.26 0.69<sup>c</sup>±0.04 1.23<sup>b</sup>±0.42 57.77<sup>a</sup>±0.76  
WHEAT

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Values are means ± standard deviation of triplicate determinations. Means with different superscripts along the same column are significantly different ( $p < 0.05$ ) according to Duncan's test.

**Key:**AYB- Bread made from 21.762 g of AYB seed coats and 78.238 g of Wheat flour; BG- Bread made from 25.577 g of BG seed coats and 74.423 g of Wheat flour; -100% wheat flour bread–Control sample

### 3.2 Total phenol content of Composite Bread

The total phenol content of the bread samples is presented in Table 2. The phenolic content of the bread was between 0.87 and 3.48 mg/g. There was a significant ( $p < 0.05$ ) increase in the phenolic content of the bread samples with addition of the pretreated seed coat. The result indicated that addition of African yam bean and Bambara groundnut seed coat flours to wheat flour significantly ( $p < 0.05$ ) increased the phenolic content of bread, with African yam bean composite bread having the highest phenolic value of 3.48 mg/g. In contrast, 100% wheat bread had the lowest phenolic value (0.87 mg/g). Increase in phenolic content of the bread could be attributed to pre-treatment of the seed coat with 2 % sodium bicarbonate. Sodium Bicarbonate is used to aid in the extraction of total polyphenols, its main effect is to increase the pH of extraction solution [36]. [37] reported that the antioxidant activity is directly proportional to the total phenol. The higher the phenolic content in a product, the higher its antioxidant activity. This observation explains the higher antioxidant activity values recorded for the composite bread samples in Table 2. The phenol contents recorded in the present study are lower than 32.72 to 71.79 mg/g earlier reported by [38] for bread from wheat-Bambara groundnut flour blends.

### 3.3 Antioxidant Properties of Composite Bread

The DPPH (2, 2-Diphenyl-1-picrylhydrazyl) values of the bread samples (Table 2) varied between 3.07 and 18.88%. The result showed that addition of African yam bean and Bambara groundnut seed coat flours to wheat flour significantly ( $p < 0.05$ ) increased the DPPH values of the bread samples. [39] also made a similar observation, reporting that addition of legume flour (African yam bean and Bambara groundnut) increased the antioxidant content of the composite flour samples. African yam bean seed coat composite bread had the highest DPPH value (18.88%) which was significantly different ( $p < 0.05$ ) from the value recorded for bread containing Bambara groundnut seed coat (5.89%), reason for the increase may be attributed to increase in DPPH of Pre-treated African yam bean seed coat by 2 % Sodium bicarbonate. Meanwhile, 100% wheat bread had the lowest DPPH. The DPPH values recorded in the present study are lower than 41.16 to 87.23%, as [18] reported for maize–Bambara groundnut composite bread. Lower amount of hydrogen could be responsible for low DPPH, [40]. [41] had earlier reported that DPPH is insoluble in water. DPPH selectively reacts with radicals and hydrogen atom donors at different reaction sites. Radicals usually attack the phenyl ring, while hydrogen donors reacts with the divalent nitrogen atom.

The ABTS values of the bread samples (Table 2) varied between 0.001 to 0.004%. Addition of African yam bean and Bambara groundnut seed coat flours to wheat flour increased the ABTS in the composite flour samples. African yam bean seed coat composite bread had the highest ABTS value (0.004 %) which was

significantly ( $p < 0.05$ ) different from (0.001 %) recorded for bread sample made from 100% wheat flour. ABTS is a very useful index used for measuring antioxidant capacity in a variety of foods [42]. A diet high in antioxidants may reduce the risk of many diseases (including heart disease and certain cancers). Antioxidants scavenge free radicals from the body cells and prevent or reduce the damage caused by oxidation [43]. The ABTS values recorded in the present study were found to be lower than 0.04 to 0.08 reported by [44] for breads from wheat-pumpkin flour blends. Low values in ABTS reported in this study could be attributed to pre-treatment of the seed coats, this conforms to the report of [45], pre-treatment reduced ABTS radical scavenging activity, possibly due to the destruction of the protein structure.

**Table 2. Total phenol and antioxidant activity of Bread containing African yam bean seed coat and Bambara groundnut seed coat.**

Sample	Total Phenol (mg GAE/g)	DPPH (%)	ABTS (Mmol/g)
AYB	3.48 <sup>a</sup> ±0.00	18.88 <sup>a</sup> ±1.86	0.004a±0.00
BGN	1.41 <sup>b</sup> ±0.35	5.89 <sup>b</sup> ±0.06	0.003a±0.00
100%WHEAT	0.87 <sup>c</sup> ±0.05	3.07 <sup>c</sup> ±1.34	0.001b±0.00

Values are means ± standard deviation of triplicate determinations. Means with different superscripts along the same column are significantly different ( $p < 0.05$ ) according to Duncan's test.

**Key:**AYB- Bread made from 21.762 g of AYB seed coats and 78.238 g of Wheat flour; BG- Bread made from 25.577 g of BG seed coats and 74.423 g of Wheat flour; -100% wheat flour bread–Control sample

### 3.4 Dietary Fibre Content of the Composite Bread

Dietary fibre content of samples is presented in Table 3. The insoluble fibre values of composite and pure wheat bread varied between 1.40 and 9.28%. The insoluble fibre content of the different bread samples was significantly different ( $p < 0.05$ ) from one another. The composite bread samples were found to have higher insoluble fibre content than the control (100% wheat bread), with African yam bean bread having the highest insoluble fibre (9.28%). The result showed that African yam bean could be a better source of insoluble fibre if added to white bread. Pre-treatment of the seed coats with 2 % Sodium Bicarbonate also contribute to the increase in the insoluble fibre content of the bread. Insoluble fibre helps to hydrate and move waste through the intestines, which helps prevent constipation [46]. The insoluble fibre values recorded in the present study are higher than 0.26 to 1.48%, as [47] reported for composite bread from a wheat-millet flour blend.

The soluble fibre values of the composite and pure wheat bread varied between 1.34 and 4.47%. Bambara groundnut composite bread was observed to have the highest soluble fibre (4.47%), while 100% wheat flour had the lowest value (1.34%). Pre-treating Bambara groundnut seed coats with 2 % Sodium Bicarbonate increased its soluble fibre which is an added advantage in the high soluble fibre recorded for its bread sample. Soluble fibre retains water and turns to gel during digestion. This action slows digestion and nutrient absorption from the stomach and intestine, thereby increasing satiety [48]. [49] also reported that increased consumption of dietary fibre content significantly reduces obesity. The dietary fibre results suggest that using Bambara groundnut seed coat flour in bread production could increase the dietary fibre of the bread product.

**Table 3. Dietary fiber content of composite Bread containing African yam bean and Bambara groundnut seed coat**

Sample	Insoluble fiber (%)	Soluble fiber (%)	Total dietary (%)
AYB	9.28 <sup>a</sup> ±0.82	3.36 <sup>b</sup> ±0.00	12.64 <sup>a</sup> ±0.00
BGN	8.26 <sup>b</sup> ±0.82	4.47 <sup>a</sup> ±0.00	12.73 <sup>a</sup> ±0.00
100% WHEAT	2.91 <sup>c</sup> ±3.42	1.34 <sup>c</sup> ±0.99	2.74 <sup>b</sup> ±4.94

Values are means ± standard deviation of triplicate determinations. Means with different superscripts along the same column are significantly different ( $p < 0.05$ ) according to Duncan's test.

**Key:**AYB- Bread made from 21.762 g of AYB seed coats and 78.238 g of Wheat flour; BG- Bread made from 25.577 g of BG seed coats and 74.423 g of Wheat flour; -100% wheat flour bread–Control sample

### 3.5 Anti-diabetic Properties (Glycemic index, alpha-amylase and alpha- glucosidase Inhibition) of Composite Bread

The glycemic index values of the composite bread samples and control (Table 4) varied between 21.85 and 53.54%. Pure wheat flour bread had the highest glycemic index (53.54%), African yam bean seed coat bread had (25.12 %) while Bambara groundnut seed coat bread had the lowest glycemic index (21.85%). It was noticed that African yam bean and Bambara groundnut seed coat significantly ( $p < 0.05$ ) reduced the glycemic index of the bread. The study showed that 100% wheat flour bread had relatively significant ( $p < 0.05$ ) high glycemic index. The glycemic index result suggests that African yam bean and Bambara groundnut seed coat bread could be recommended to diabetic patients since they have a low glycemic index. The glycemic index measures how much specific foods increase blood sugar levels [50]. [51] reported that consuming a medium glycemic index food may help improve glycemia. Low GI has been associated with a lower risk of developing type 2 diabetes and coronary heart disease and may help individuals with insulin resistance [52]. WHO/FAO recommends low-GI foods to prevent lifestyle-related diseases [50]. The glycemic index values recorded in the present study are lower than 51.42 to 72.80 reported by [53] for wheat-orange fleshed sweet potato composite bread.

The Alpha-amylase inhibition values of the composite bread and control sample (Table 4) varied between 26.99 and 93.31%. African yam bean seed coat bread had the highest Alpha-amylase inhibition, while 100% wheat bread had the lowest value. Significant differences ( $p < 0.05$ ) were observed in the alpha amylase inhibition of the different bread samples. The result showed that incorporation of African yam bean and Bambara groundnut seed coat flour into bread increased the Alpha amylase inhibition of the bread samples. Alpha-amylase inhibitors can act as carbohydrate blockers, limiting the digestibility and absorption of carbohydrates in the gastrointestinal diet [54]. Clinically,  $\alpha$ -amylase inhibitors can prevent diabetes, hyperglycemia, hyperlipemia and obesity [54]. The alpha-amylase inhibition values recorded in the present study are higher than 29.96 to 56.77% reported by [55] for bread from *Sphenostylisstenocarpa*-wheat composite flour and 51.7 to 81.0% reported by [56] in bread from Bambara groundnut composite flour. The higher values recorded in this study might be associated to the high content of dietary fibre in the seed coats used in the composite flour.

The Alpha-glucosidase inhibition values of the composite bread and the control sample (Table 4) varied between 34.21 and 74.44%. African yam bean seed coat bread had the highest Alpha-glucosidase inhibition

value while 100% wheat bread had the lowest Alpha-glucosidase inhibition value. The addition of seed coat to wheat flour caused significant increase ( $p < 0.05$ ) in the Alpha-glucosidase inhibitory activity of the different bread samples. The result showed that African yam bean seed coat bread had a higher Alpha-glucosidase inhibition effect than pure wheat flour bread. Hence, adding African yam bean seed coat flour to wheat flour could help increase the Alpha-glucosidase activity of bread, which can be recommended for diabetic patients. Alpha-glucosidase inhibitors reduce the absorption of carbohydrates from the small intestine. They competitively inhibit enzymes that convert complex, non-absorbable carbohydrates into simple, absorbable carbohydrates. These enzymes include glucoamylase, sucrase, maltase, and isomaltase [57]. The Alpha-glucosidase inhibitory activity values recorded in the present study are higher than 14.21 to 22.50%, as [58] reported for bread from wheat, whole millet, and natural hydrocolloid composite flour.

Comment [UG9]: Good work

**Table 4. Anti-diabetic properties of composite Bread containing African yam bean and Bambara groundnut seed coat**

Sample	Glycemic Index	Alpha-amylase inhibition (%)	Alpha-glucosidase inhibition (%)
AYB	25.12 <sup>a</sup> ±0.05	93.31 <sup>a</sup> ±0.13	77.19 <sup>a</sup> ±0.00
BGN	21.85 <sup>c</sup> ±0.05	88.89 <sup>a</sup> ±0.25	74.44 <sup>b</sup> ±2.63
100% WHEAT	53.54 <sup>b</sup> ±0.14	26.99 <sup>c</sup> ±0.13	34.21 <sup>c</sup> ±2.63

Values are means ± standard deviation of triplicate determinations. Means with different superscripts along the same column are significantly different ( $p < 0.05$ ) according to Duncan's test.

**Key:**AYB- Bread made from 21.762 g of AYB seed coats and 78.238 g of Wheat flour; BG- Bread made from 25.577 g of BG seed coats and 74.423 g of Wheat flour; -100% wheat flour bread–Control sample

### 3.6 Sensory Attributes of Composite Bread containing African yam bean, Bambara groundnut seed coat.

Table 5 presents the sensory characteristics of the composite bread made from African yam bean, Bambara groundnut seed coatings, and the control sample. The crust colour, as determined by the panellists, exhibited a substantial variation ( $p < 0.05$ ) in the scores given by the panellists (ranging from 6.45 to 7.68). The study showed a marginal preference for the crust colour of the two composite breads, but a high preference for the control sample. The mean score for crumb color ranged from 5.64 to 7.53. The crumb colour of bread made with African yam bean seed coat was somewhat preferred, whereas bread made with Bambara groundnut seed coat and pure wheat flour bread were considerably preferred. The appearance scores ranged from 5.76 to 7.27. The African yam bean seed coat bread exhibited a mild preference, whereas the Bambara groundnut seed coat bread and the control sample demonstrated a moderate preference. The AYB seed coat's pigmentation may have influenced the composite bread's reduced rating. The elevated fiber content in the composite bread contributed to its darker look and crumb colour compared to the control sample [59]. The composite bread exhibited a pleasant aroma and flavour, which were found to be well-received than the control sample, as evidenced by their sensory scores. The seed coat's presence improves the bread's fragrance and flavour [60]. The average sensory score for scent ranged from 6.56 to 6.91. The three samples' fragrance received moderate appreciation. The bread texture scores ranged from 6.35 to 7.45. The composite bread samples were somewhat favourable, whereas the control sample was highly avorable. The taste scores ranged from 6.09 to 6.75. The panellists showed a considerable level of liking for the bread containing African yam bean

and Bambara groundnut seed coat, whereas the control sample received only moderate appreciation. The panellists rated the bread samples, ranging from 6.50 to 7.00, for overall acceptability. The panellists rated both the composite bread and control sample as moderately liked in terms of overall acceptability. Remarkably, there was no significant difference ( $p > 0.05$ ) in the overall acceptability between the composite bread and the control sample. The panel members' preference may have been impacted by their familiarity with bread baked exclusively from wheat flour. These observations corroborate the findings of [61] regarding the sensory attributes of sorghum-based bread.

**Table 5. Sensory attributes of composite bread containing African yam bean and Bambara groundnut seed coat**

Attribute	Samples		
	AYB bread	BGN bread	Pure wheat bread
Crust Color	6.45 <sup>c</sup> ± 1.93	6.97 <sup>b</sup> ± 1.14	7.68 <sup>a</sup> ± 1.56
Crumb Color	5.64 <sup>b</sup> ± 1.86	7.16 <sup>a</sup> ± 1.10	7.53 <sup>a</sup> ± 1.41
Appearance	5.76 <sup>b</sup> ± 1.86	7.33 <sup>a</sup> ± 1.08	7.27 <sup>a</sup> ± 1.65
Aroma	6.56 <sup>a</sup> ± 2.07	6.76 <sup>a</sup> ± 1.58	6.91 <sup>a</sup> ± 1.59
Texture	6.35 <sup>b</sup> ± 1.67	6.66 <sup>b</sup> ± 1.33	7.45 <sup>a</sup> ± 1.67
Taste	6.75 <sup>a</sup> ± 1.56	6.95 <sup>a</sup> ± 1.58	6.09 <sup>b</sup> ± 1.98
Overall Acceptability	6.50 <sup>a</sup> ± 1.69	7.00 <sup>a</sup> ± 1.05	6.94 <sup>a</sup> ± 1.58

Values are means ± standard deviation of triplicate determinations. Means with different superscripts along the same column are significantly different  $p < 0.05$  according to Duncan's test.

**Key:**AYB- Bread made from 21.762 g of AYB seed coats and 78.238 g of Wheat flour; BG- Bread made from 25.577 g of BG seed coats and 74.423 g of Wheat flour; -100% wheat flour bread–Control sample

#### 4. CONCLUSION

This study has indicated that pre-treated seed coats of African yam bean and Bambara groundnut with 2% sodium bicarbonate could be successfully utilised in the formulation of composite flour for the production of functional bread. The composite bread had noticeable increase in fibre, protein, ash contents, total phenol and antioxidant properties as well as soluble and insoluble dietary fibre. The anti-diabetic properties were also improved while the bread also had acceptable sensory properties. These characteristics are good pointers to the possible utilisation of underutilised legume seed coats in different food applications, thus widening their utilisation.

**Comment [UG10]:** This study has lot more significance in food industry as well as in the management of metabolic disorders. I would suggest to mention about their future implications.

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Comment [UG11]: Relevant ??

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