European Journal of Nutrition & Food Safety

Volume 16, Issue 5, Page 32-47, 2024; Article no.EJNFS.115687
ISSN: 2347-5641

Amino Acid Compositions, *In Vitro* Antioxidant and Anti-diabetic Properties of Cookies from Wheat and Kidney Beans (*Phaseolus vulgaris* L.) Flour blends

Dele Ayorinde Olowookere [a] and Sunday Abiodun Malomo [b*]

[a] Department of Nutrition and Dietetics, Ekiti State College of Health Sciences and Technology, Ijero, Nigeria.
[b] Department of Nutrition and Dietetics, Federal University of Technology, Akure, Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJNFS/2024/v16i51420

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/115687

Original Research Article

Received: 11/02/2024
Accepted: 15/04/2024
Published: 23/04/2024

ABSTRACT

This study evaluated the *in vitro* antioxidant and antidiabetic activities of the cookies produced from wheat and kidney bean composite flours at different ratios *viz:* 100:0 (WKB 1), 80:20 (WKB 2), 60:40 (WKB 3), 40:60 (WKB 4), respectively. The proximate compositions of the composite flour blends improved during the baking process into cookie, most especially the crude fibre (12.09-13.73%) and crude protein (18-21%) contents, respectively. The amino acid profiles of the cookies were well established with high biological values (>70%) with good essential, non-

**Corresponding author: Email: samalomo@futa.edu.ng;*

essential and hydrophobic amino acids while glutamic acid was mostly abundant in the cookies. The *in-vitro* antioxidant properties of the cookie samples were more potent (~80%) when compared with a standard ascorbic acid, a well-known antioxidant. Besides, the *in-vitro* anti-diabetic properties of the cookie samples were revealed through their improved α-amylase and α-glucosidase inhibition potentials (~70%) when compared with a standard acarbose, a well-known anti-diabetic drug. The weights (5.20-6.41 g), width (45.70-45.88 mm), thickness (5.03-5.06 mm) and spread ratio (11.89-12.26) of the cookies from the composite flours were significantly ($P<0.05$) comparable to the control (WKB 1) sample, respectively. This, however did not alter the organoleptic attributes of the composite cookie samples when compared to the commercial ones. We therefore concluded that the cookies rich in antioxidants and anti-diabetic potentials could be produced from wheat and kidney bean flour blends.

Keywords: kidney bean; germination; amino acid; anti-inflammatory; cookies.

1. INTRODUCTION

Diabetes is a fast-growing illness in our society which implied the high rate of sugar level in the blood and could thus, resulted in heart damage [1]. Meanwhile, this condition could be reduced or totally eradicated by consuming a high antioxidant food with resultant anti-diabetic potentials [2]. “Nutritional therapy has been a current and preferably means in the management of metabolic diseases such as diabetes mellitus (DM) instead of synthetic drugs that hardly ameliorated extra-pancreatic degenerations, which often resulted from diabetic conditions” [1]. “Therefore, the increasing awareness and scientific evidence clearly indicated a strong relationship between health and diet. These factors have generated new concept for researchers in the field of nutrition targeting the development and promotion of functional foods. The rapid increase in snacks consumption, health awareness and demand for nutritious foods have necessitated researchers on composite cookies to meet these needs” [3].

“Free radicals have been implicated to cause oxidative stress in the disease pathogenesis and management systems, which gave rise to unacceptable state of health that reduced the quality of life through development of several chronic diseases” [4]. “Therefore, it is important to inhibit or scavenge these radicals by using antioxidants. Oxidative stress seemed to play a significant role in many human diseases, including cancers. The use of antioxidants in pharmacology is intensively studied, particularly as treatments for stroke and neurodegenerative diseases. For these reasons, oxidative stress could be considered to be both the cause and the consequence of some diseases” [5]. “Antioxidants worked by chain breaking e.g., α-tocophenol, reducing the concentration of reactive oxygen species e.g. glutathione, scavenging initial radicals e.g. superoxide dismutase, which acted in aqueous phase to trap superoxide free radicals and chelating the transition metal catalyst; a group of compound serving as an antioxidant function by sequestration of transition metals that were well established pro-oxidants. Although, the body possessed its natural self-defense mechanisms (such antioxidants like gluthathione and superoxide dismutase) to fight the free radicals, but there existed a concern about the ineffective amount being released during illness and old age developments” [6].

“Kidney bean has been chosen for this work because its structural properties as related to its albumin and globulin fractions have been properly characterized” [7]. “For example, kidney bean globulins had higher arginine and branched chain amino acid (BCAA) than the albumin whereas the globulin was very poor in sulfur-containing amino acids (SCAA)” [7]. “These differences in amino acid composition can be exploited for health specific health benefits. For instance, positive cardiovascular disease interventions can be achieved with diets containing a high arginine/lysine” [8]. “Similarly, diets with high SCAA or specifically cysteine contents may also be desirable for cardiovascular benefits since this amino acid is a critical structural component of glutathione, the highly potent cellular antioxidant. Meanwhile, a low cellular glutathione levels have been associated with poor health status and disease development” [9]. Therefore, this work used a mechanistic approach by taking advantage of the amino acid differences between the kidney bean albumin and globulin protein fractions to test efficacy as blood sugar-reducing agents. Besides, kidney beans have long had a long history of consumption by human being without

being toxic to them. Hence, the protein, amino acid and other nutrients present in the cookies would aid the effective anti-diabetic properties of the cookies. People living with diabetes -related health-issues would benefit from the knowledge of usefulness of natural plant-based snacks and caused less dependence on expensive synthetic products. This is because synthetic drugs like acarbose are laced with expensive costs and negative side effects such as nausea, vomiting, etc.

"The composite flour from kidney beans and wheat flour is considered advantageous in the developing countries as it reduced the importation of wheat flour and encouraged the use of locally grown crops" [1]. The strong attributes of the cookies produced from the sprout of kidney beans and wheat composite flour compared favourably with that 100% wheat flour [10]. Therefore, the cookies from indigenous crop like kidney bean with low amount of wheat flour is expected to meet the demand of most coeliac and diabetic patient [11]. This study, thus aimed to investigate the *in vitro* antioxidant and antidiabetic activities of the cookies produced from wheat and kidney bean composite flours.

2. MATERIALS AND METHODS

2.1 Materials

The commercial wheat flour, which have been commonly used for all baking processes, was obtained from a commercial baking ingredients store in Ikere Ekiti, Nigeria. The kidney bean seeds were obtained from the King's market, Ikere Ekiti, Nigeria and authenticated at the Department of Crop, Soil and Pest Management, Federal University of Technology, Akure, Nigeria. All chemicals used were of analytical grade and obtained from Sigma-Aldrich, London, United Kingdom.

2.2 Preparation of Kidney Bean Flour

"The germinated kidney bean flour was obtained according to the method previously described" [12]. "Briefly, the kidney beans were soaked in water for 2 h to achieve hydration, after which it was then left at room temperature (25 ^{o}C) for 48 h. This was thereafter rinsed and spread on a jute bag for germination to take place while the seeds were closely monitored frequently, frequently watered and separated so as to prevent mold growth. After 48 h, the germination process was terminated by exposing the seeds to a temperature of 40 ^{o}C for 3 h and milled into flour. The resulting flour was defatted using n-hexane in soxhlet extraction apparatus as described" [13]. The defatted sample was air-dried in a fume hood at room temperature to drive off the n-hexane completely, blended and thereafter sieved and preserved in tightly closed plastic container pending analysis.

2.3 Formulation of Composite Flour Blends

The composite flour blends were formulated from wheat and kidney bean flours in the following ratios *viz:* 100:0, 80:20, 60:40, 40:60 (wheat flour:kidney bean flour) and depicted as WKB 1, WKB 2, WKB 3 and WKB 4, respectively.

2.4 Production of Cookies

Cookies were produced as previously described [14] with the following ingredients, composite flour, margarine, baking powder, salt, beet, eggs and water. The dry ingredients were thoroughly mixed in a bowl for few minutes followed by adding the margarine and eggs and kneaded to form batter. The batter was then rolled on a rolling board sprinkled with flour for a uniform thickness and cut with a 50 mm-diameter cookie cutter. The cookies were placed in baking trays leaving a 25 mm space in between and baked at 180^{0}C for 10 min in the baking oven. After baking, the cookies were cooled at ambient temperature, packaged in polyethylene bags and stored prior to subsequent analysis.

2.5 Proximate Composition Analysis of the Composite Flours and Cookies

"The proximate composition (moisture content, crude fiber, crude fat, total ash, and crude protein contents of the flour blends and cookies was determined as described" [13]. "The total carbohydrate content was obtained by difference. The crude protein contents were determined by micro-Kjedahl method to obtain the nitrogen content. The crude protein was then calculated as (gN x 6.25) while the crude fat was obtained using Soxhlet apparatus" [13].

2.6 Amino Acid Analysis of the Cookies

"The amino acid profiles of the cookies were determined using the modified High-performance liquid chromatography (HPLC) method as

previously described" [15]. **"**The samples were derivatized for 20 min using a solvent mixture containing 95% ethanol: water: triethylamine: phenylisothiocyanate (7:1:l:1), dried under vacuum and dissolved in buffer A prior to HPLC separation on the Pico-Tag column using a flow rate of 0.45 mL/min and detection at 254 nm. The gradient was from 10-50% buffer B (60% acetonitrile and 40% water by volume) in buffer A (940 mL of 0.14 M sodium acetate, pH 6.40, containing 0.05% triethylamine, mixed with 60 mL acetonitrile) over 10 min" [13].

2.6.1 Calculated nutritional quality determinations

The Biological Value (BV) was calculated according to the method described [16] using the following equation: BV = 1.09 x Essential amino acid index [EAAI] - 11.7.

2.7 Antioxidant Assays of the Cookies

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

"The radical scavenging activities of the cookie samples were determined using the stable radical DPPH (2,2-diphenyl-1-picrylhydrazyl hydrate) as described" [15]. The reaction of DPPH with an antioxidant compound which can donate hydrogen, leads to its reduction. The change in colour from deep violet to light yellow was measured spectrophotometrically at 517 nm. To 1 ml of different concentrations of the extract or standard (ascorbic acid) in a test tube was added 1 ml of 0.3 mM DPPH in methanol. The mixture was mixed and incubated in the dark for 30min after which the absorbance was read at 517 nm against a DPPH control containing only 1ml methanol in place of the extract.

$$\%\ \text{Radical Scavenging Activity} = \left[\frac{(A_{blank} - A_{sample})}{A_{blank}}\right] X100$$

Where A_b is the absorbance of blank and A_s the absorbance of the extract.

2.7.2 Determination of Ferric Reducing Antioxidant Power (FRAP) of the cookies

"Ferric reducing antioxidant power was determined as described" [15]. "Briefly, 100 µl of the extract were mixed with 2.5 ml of 200 mmol/l phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide and incubated at 50 °C for 20 min. Then, 2.5 mlof 10% trichloroacetic acid was added, and the tubes were centrifuged at 10,000 rpm for 10 min. After this, 5 ml of the upper layer were mixed with 5.0 ml distilled water and 1 ml of 0.1% ferric chloride, and the absorbance of the reaction mixtures was measured at 700 nm. Ascorbic acid was used as a positive control" [15].

2.7.3 Determination of hydroxyl radical scavenging activity of the cookies

The deoxyribose assay was used to determine the hydroxyl radical scavenging activity in an aqueous medium according to the procedures previously described [15]. The reaction mixture containing $FeCl_3$ (100 µM), EDTA (104 µM), H_2O_2 (1 mM) and 2-deoxy- D-ribose (2.8 mM) at various concentrations of extracts in 1 ml final reaction volume made with potassium phosphate buffer (20 mM, pH 7.4) and incubated for 1 hr at 37^{o}C. The mixture was heated at 95 ^{o}C in water bath for 15 min followed by the addition of 1 ml each of TCA (2.8%) and TBA (0.5% TBA in 0.025 M NaOH containing 0.02% BHA). Finally, the reaction mixture was cooled on ice and centrifuged at 5000 rpm for 15 min. Absorbance of supernatant was measured at 532 nm. Ascorbic acid was taken as the positive control.

$$\%\ \text{Hydroxyl Scavenging Activity} = (A_c - A_s) / A_c * 100$$

Where A_c is the absorbance of control and A_s the absorbance of the extract.

2.7.4 Determination of iron chelating activity of the cookies

Metal chelating activity was measured as described [15]. 0.1 mM $FeSO_4$(0.2 ml) and 0.25 mM ferrozine (0.4 ml) were subsequently added into 0.2 ml of flour sample and dough meal. After incubating at room temperature for 10 min, absorbance of the mixture was recorded at 562 nm. Chelating activity was calculated using the following formula:

$$\text{Metal Chelating Activity} = \left[\frac{(A_{control} - A_{sample})}{A_{control}}\right] X100$$

Where $A_{control}$ is the absorbance of control reaction (without plant extract), and A_{sample} is the absorbance in the presence of a plant extract.

2.8 Inhibition of α-Amylase Activity Assay of the Cookies

"The *in-vitro* α-amylase activity could be measured by hydrolysis of starch in presence of α-amylase enzyme. This process was quantified by using iodine, which gave blue colour with starch. The reduced intensity of blue colour indicated the enzyme-induced hydrolysis of starch in to monosaccharides. If the substance possessed α-amylase inhibitory activity, the intensity of blue colour would be more. In other words, the intensity of blue colour in test sample is directly proportional to α-amylase inhibitory activity" [17]. α-amylase activity was carried out by starch-iodine method. 10 µL of α- amylase solution (0.025 mg/ml) was mixed with 390 µl of phosphate buffer (0.02 M containing 0.006 M NaCl, pH 7.0) containing different concentration of extracts. After incubation at 37 °C for 10 min, 100 µl of starch solution (1%) was added, and the mixture was re-incubated for 1 h. Next, 0.1 ml of 1% iodine solution was added, and after adding 5 ml distilled water, the absorbance was taken at 565 nm. Sample, substrate and α-amylase blank determinations were carried out under the same reaction conditions. Inhibition of enzyme activity was calculated as (%) = (A-C) X100/ (B-C), where, A= absorbance of the sample, B= absorbance of blank (without α-amylase), and C= absorbance of control (without starch).

2.9 Inhibition of α-Glucosidase Activity Assay of the Cookies

The α-glucosidase inhibition activity was performed according to the slightly modified method [17]. The α-glucosidase activity can be measured in-vitro by determination of the reducing sugar (glucose) arising from hydrolysis of sucrose by α-glucosidase enzyme. The final volume of the reaction mixture was 100 µl, which contained 70 µl of phosphate buffer saline (50 mM, pH 6.8), 10 µl of test extracts, and 10 µl (0.057 U) enzyme. The content was mixed, pre-incubated at 37ºC for 10 min, and pre-read against the reagent blank value by spectrophotometry at 400 nm. The reaction was initiated using 10 µl of 0.5 mM substrate (i.e., p-nitrophenol glucopyranoside). Acarbose was used as a positive control. After incubation at 37ºC for 30 min, optical absorbance was measured against the reagent blank value by spectrophotometry at 400 nm. The percentage of enzyme inhibition was calculated using the Equation.

$$\%\ \text{Enzyme inhibition Activity} = \left[\frac{(A_c - A_s)}{A_c}\right] X100$$

Where Ac is the absorbance of control and As the absorbance of the extract.

2.10 Determination of Physical Properties of Cookies

The cookies were analysed for weight, diameter, thickness (width) and spread factor (diameter/thickness) according to respective procedures previously described [14]. Cookie diameter (D) and thickness (T) were determined using a vernier caliper. Spread factor (SF) was also determined from the diameter and thickness, using a formula:

SF = (D/T X CF) X 10 where CF is correction factor, at constant atmospheric pressure.

2.11 Evaluation of Sensory Attributes of Cookies

The cookies were coded and presented to twenty (20) semi-trained panelists to be evaluated for their appearance, texture, taste, aroma, mouth feel, crumblings, overall acceptability using the Hedonic scale of 1 to 9, where 1 = dislike extremely and 9 = like extremely as previously described [14].

2.12 Statistical Analysis

All determinations were carried out in triplicates. Data was subjected to analysis of variance (ANOVA) using SPSS (version 21, USA), while means was separated using New Duncan Multiple Range Test (NDMRT) at 5% level of significance ($p<0.05$).

3. RESULTS AND DISCUSSION

3.1 Proximate Compositions of Flour Blends and Cookies

The moisture contents (m.c.) of all the flour samples, presented in Table 1, ranged between 5.22 and 6.33% with significant ($P<0.05$) highest and lowest m.c. in samples WKB2 and WKB1, respectively. Moisture is an important factor in food quality, preservation and resistance to deterioration [18]. The implication of this low m.c. therefore is that the samples have a tendency of high shelf stability over a period of time. Interestingly, all the flour samples fall under the

FAO recommended m.c. of <10% for most viable dried food products [19]. The protein contents in the flour samples ranged from 13.09 to 28.03% with WKB1 having the lowest while WKB4 had the highest content, respectively. The crude fats in the flour samples ranged from 1.24 to 1.71% with WKB4 having the lowest presence of fats while WKB1 had the highest presence of fats. The total ash in the flour samples ranged between 1.62 and 3.23% with WKB1 having the least and WKB4 having the highest values, respectively. The determination of ash is an important quality attribute for some food ingredients used to measure total amount of minerals present within a food [3]. Hence, the high values reported for the composite flours, when compared with the WKB1, revealed that the composite flours would contain high amount of nutritionally important minerals. The crude fibre in the flour samples ranged from 1.86 to 4.28% with WKB1 having the lowest and WKB4 having the highest. The result revealed that the fibre contents increased as the level of the kidney beans flour substitution increased. The carbohydrate contents ranged from 60.41 to 83.31% in the flour samples with WKB4 being the lowest and WKB1 being the highest. The low carbohydrate content in WKB4 might be due to its high protein contents, when compared to other flour samples.

Table 2 showed the proximate compositions of cookies samples made from wheat-kidney bean flour blends. The moisture content (m.c.) of the cookie samples ranged from 7.02 to 8.01%. Accordingly, the samples WKB1 had the significant ($P<0.05$) highest m.c. while the m.c. of the remaining composite products, WKB2, WKB3 and WKB4, were not differed significantly ($P>0.05$). The protein contents of the cookie samples ranged from 11.82 to 21.87% with WKB1 being the lowest and WKB4 being the highest. The values agreed with the past work [3], which indicated the fortification process increased the protein content while producing a shelf stable product at the same time, since legumes have higher protein content than cereals as revealed in sample WKB4. The protein contents of the cookies sample were lower than that of the flour samples and this implied that baking reduced protein content because of gluten-starch complex formation during baking [3]. The crude fats in the cookie samples ranged from 5.12 to 7.13% with WKB2 and WKB1 having the lowest and highest values, respectively. The current results are expected based on the previous finding [10] that had shown the kidney beans to possess low levels of lipid. The total ash in the cookie samples ranged between 4.06 and 4.35% with WKB1 having the least value and WKB4 having the highest value, respectively. WKB1 had the least value of total ash because it had no kidney beans, which were present in other samples and kidney bean was known to be rich in macro elements such as calcium, potassium, sodium and iron [10]. The fibre contents in the cookie samples ranged from 7.04 to 14.21% with WKB1 having the lowest and WKB3 having the highest, which showed that the introduction of kidney bean flours increased the fibre contents of the composite flours. Crude fibre also helped to prevent heart diseases, colon cancer, diabetes etc., reduced the rate of glucose into blood stream and inter-colonic pressure thereby reducing the risk of colon cancer [20]. The carbohydrate contents of the cookie samples ranged from 57.29 to 71.44% with WKB3 and WKB1 having the lowest and highest values, respectively. Consumption of these samples can be effective in the regulation of blood sugar because of its low carbohydrate content that could result into estimated low glycemic index as previously observed [21].

3.2 Amino Acids Profiles of Cookies Samples

The amino acid composition of the cookie samples is presented in Table 3. Besides the various amino acids presented, the table showed the distribution of the essential amino acids (EAAs), non-essential amino acids (NEAAs), hydrophobic amino acids (HAAs) and total amino acids (TAAs) present in the different cookie samples. It has been reported that amino acids are building blocks of proteins and served as important bio-molecules that regulated key metabolic pathways as well as precursors for synthesis of biologically important substances [22]. Moreso, the essential amino acids cannot be synthesized by the body, hence are being supplied from an exogenous diet such as those available in some plant-based foods [22]. Interestingly, the most dominant amino acid in the cookies sample was glutamic acid, with the highest value (17.36 mg/100 g) in sample WKB4. Glutamic acid is crucial for brain development, essential in immunity, important for bones and also function as an anti-cancer agent [22-23]. Moreso, sample WKB4 has the highest value for total amino acids and biological value which implied that it has the best amino acid profile out of all the cookies samples and when

consumed, would be readily available and easily absorbed into the GIT system. The essential amino acids found in the present cookie samples were histidine, isoleucine, leucine, methionine, phenylalanine, threonine, tryptophan and valine with sample WKB3 having the highest value (48.04%) when compared to other samples. The non-essential amino acids, such as glutamic acid, proline, glycine and arginine, which were also synthesized in the body to meet the needs for maximal growth and health and include were present in the samples with highest amount in sample WKB4 (45.35%). The NEAAs were very important in regulating gene expression, cell signaling, anti-oxidative responses, neurotransmission and immunity [23]. However, the hydrophobic amino acids, such as alanine, valine, leucine, isoleucine, phenylalanine, methionine, tryptophan and cysteine were highly present and obtained in the sample WKB4 (34.20%) when compared to other cookies samples. This means sample WKB4 has the highest potentials to be used as bioactive agents in the modulation of pathogenesis of any disease (as antioxidant agent), especially in the management of chronic diseases. This is because the high presence of hydrophobic amino acids has been previously reported as a common factor for most bioactive compounds (like peptides, polyphenols, etc) with anti-diabetic properties [24].

3.3 Antioxidant Properties of Cookies

It is well established that antioxidants, inhibited oxidation process by preventing the formation of free radicals play major roles in preventing chronic diseases such as cardiovascular diseases, diabetes, obesity and cancers [25]. The hydroxyl (OH) radical scavenging activity of the cookie samples is presented in Fig 1. The properties of the samples were recorded in the ranges of 23 to 78% when compared to the common and well-known antioxidant, ascorbic acid (80%). It was observed that the sample WKB4 had the significant ($p<0.05$) highest property when compared to sample WKB1 (cookie from wheat flour only). The DPPH radical scavenging activity of the samples is obtained in the following ranges; 30.42-80.16% as shown in Fig 2. It was observed that the samples shown significant ($p<0.05$) difference between each other against DPPH activities and when compared to ascorbic acid. It was also observed that the cookie samples were significantly ($p<0.05$) less than ascorbic acid in free radical scavenging activities. A similar trend of low Fe^{2+} chelation antioxidant activity was observed in WKB1 (28%) when compared to WKB4 (78%) as presented in Fig. 3. It was observed that the antioxidant activities of sample WKB1, 2 and 3 (with respect to hydroxyl, DPPH radical scavenging and Fe^{2+} chelation antioxidant activities) were significantly ($p<0.05$) lower than WKB4. This implied that the samples WKB1, 2 and 3 exhibited less ability to scavenging free radicals against hydroxyl and DPPH radicals thereby having less potential to chelate Fe^{2+}. The result presented in Fig. 4 revealed that the sample WKB4 also had highest ferric reducing antioxidative potentials (FRAP) when compared to others. For instance, WKB4 and WKB1 had 0.82 and 0.50 mmol Fe^{2+}/mg when compared with ascorbic acid (0.90 mmol Fe^{2+}/mg). The relative higher antioxidant potentials (~80%) of the cookie sample WKB4 may be attributed to its higher protein content (21.87 mg/100 g), hydrophobic amino acids (34.20 mg/100 g) and biological values (86.28%) when compared to other samples. This observation agreed with other findings, that reported the efficacy of protein and amino acids profiles to enhance the antioxidant capacity, which was related to the release of bioactive peptides [26]. This finding also agreed with past study that reported on the contributions of antioxidants in diabetes and its complications [27]. Several studies have demonstrated significant decrease of cardiovascular disease such as diabetes and hypertension with consumption of food rich in antioxidants [27-29]. Hence, sample WKB4 could help in reducing the risk of cardiovascular diseases.

3.4 Anti-diabetic Properties (α-amylase and α-Glucosidase Inhibition potentials) of Cookies

The α-amylase is a prominent enzyme found in the pancreatic juice and saliva that hydrolyzed complex starches to oligosaccharides while α-glucosidase is the enzyme found in the mucosal brush border of the small intestine that hydrolyzed oligosaccharides to glucose and other monosaccharides [30-31]. Fig 5 showed the α-amylase inhibition abilities of the composite wheat-kidney bean cookie samples. The percentage inhibition of the α –amylase by the cookie samples range from 25% (WKB1) to 60% (WKB4). The cookie WKB4 had significant ($p<0.05$) higher α–amylase inhibition than the other samples (WKB1, 2 and 3) but lower than the activity obtained (65%) for acarbose, a common anti-diabetic drug. The

higher hydrophobic acid obtained for WKB4 (Table 4) may account for its higher inhibition [32]. The highest α–amylase inhibition (60%) exhibited by WKB4 could enhance its usage as a potential anti-diabetic agent with no negative side-effect as non-insulin dependent diabetic mellitus (NIDDM) diet. The results presented in Fig 6 showed the potential of the cookie samples to inhibit α- glucosidase activities. The inhibition potentials ranged from 22% (WKB1) to 50% (WKB4). The other composite cookie samples (WKB2 and WKB3) have less inhibition than the WKB4. However, the result (Fig 6) showed no significant difference ($p>0.05$) between cookie sample WKB4 and acarbose, a well-known anti-diabetic drug. Hence, the cookie sample WKB4 has potential to be used to modulate the type 2 diabetes. The cookie sample WKB4 obtained from 60% kidney bean flour has improved inhibition of α-glucosidase and this may be due to its higher protein contents as well as its better hydrophobic amino acids, when compared to other samples [32]. In the management of diabetic patients, the inhibition of α-amylase and α-glucosidase that are involved in the breakdown of carbohydrate prevented starch hydrolysis. This, thus resulted in a decreased level of glucose available for assimilation into the blood through regulation of the postprandial glycemic level [33].

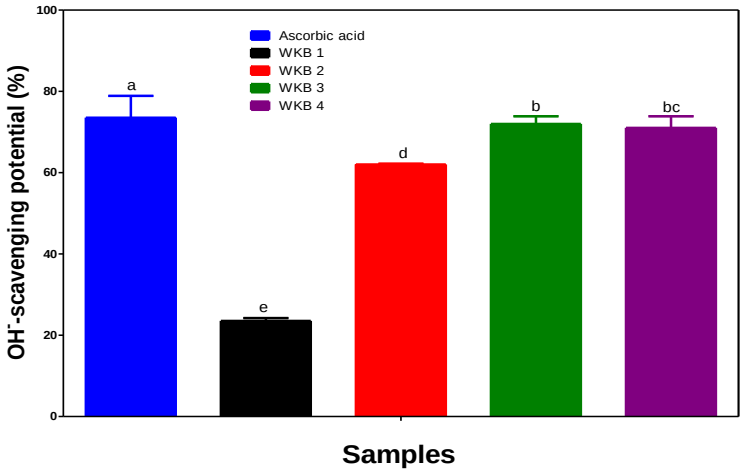

Fig. 1. Hydroxyl (OH) radical scavenging potential of different cookies samples

Bars (n=3) with different letter are significantly different ($p<0.05$), Key: WKB 1 = 100% wheat flour (Negative control); WKB 2 = 80% wheat flour + 20% kidney bean flour; WKB 3 = 60% wheat flour + 40% kidney bean; WKB 4 = 40% wheat flour + 60% kidney bean

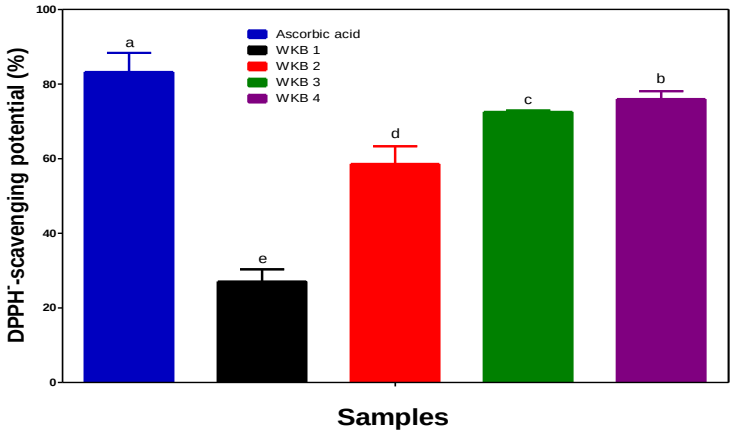

Fig. 2. DPPH radical scavenging potential of different cookies samples

Bars (n=3) with different letter are significantly different ($p<0.05$), Key: WKB 1 = 100% wheat flour (Negative control); WKB 2 = 80% wheat flour + 20% kidney bean flour; WKB 3 = 60% wheat flour + 40% kidney bean; WKB 4 = 40% wheat flour + 60% kidney bean

Table 1. Proximate composition of composite flours (%) (on dry weight basis)

Sample	Moisture	Crude fat	Crude protein	Ash	Crude fibre	Carbohydrate
WKB 1	6.33^{b} ±0.03	1.71^{a} ±0.06	13.09^{d} ±0.05	1.62^{d} ±0.01	1.86^{e} ±0.06	83.31^{a} ±0.06
WKB 2	5.22^{c} ±0.09	1.62^{b} ±0.03	20.53^{c} ±0.03	2.48^{c} ±0.02	2.65^{d} ±0.03	71.83^{b} ±0.07
WKB 3	5.24^{c} ±0.09	1.30^{c} ±0.05	26.11^{b} ±0.02	3.23^{a} ±0.08	3.61^{c} ±0.05	65.13^{c} ±0.05
WKB 4	5.23^{c} ±0.07	1.24^{d} ±0.06	28.03^{a} ±0.06	3.01^{b} ±0.01	4.28^{ab} ±0.01	60.41^{e} ±0.09
Kidney bean flour	7.22^{a} ±0.03	1.33^{c} ±0.06	26.04^{b} ±0.05	3.19^{a} ±0.01	4.35^{a} ±0.06	64.98^{d} ±0.06

Means (n=3) with different letter in the column are significantly different (p<0.05).

Key: *WKB 1 = 100% wheat flour; WKB 2 = 80% wheat flour + 20% kidney bean flour; WKB 3 = 60% wheat flour + 40% kidney bean flour; WKB 4 = 40% wheat flour + 60% kidney bean flour*

Table 2. Proximate composition of cookies samples (%)

Sample/ Parameters	Moisture	Crude fat	Crude protein	Ash	Crude fiber	Carbohydrate
WKB 1	8.01 ± 0.36^{b}	7.13±0.06^{b}	11.82 ± 0.12^{d}	4.06 ± 0.04^{b}	7.04 ± 0.30^{d}	71.44 ± 0.11^{a}
WKB 2	7.04 ± 0.06^{c}	5.12 ± 0.07^{c}	18.42 ± 0.05^{c}	4.29 ± 0.14^{a}	12.09 ± 0.06^{c}	59.38 ± 0.09^{d}
WKB 3	7.06 ± 0.05^{c}	5.15 ± 0.01^{c}	19.83±0.05^{b}	4.30±0.05^{a}	14.21 ± 0.00^{a}	57.29 ± 0.08^{e}
WKB 4	7.02 ± 0.11^{c}	5.14 ± 0.03^{c}	21.87 ± 0.01^{a}	4.35 ± 0.01^{a}	13.73± 0.05^{ab}	58.19 ± 0.14^{c}
Commercial sample	8.27 ± 0.11^{a}	10.02 ± 0.03^{a}	10.91± 0.01^{e}	3.61± 0.01^{c}	4.72 ± 0.05^{e}	72.69 ± 0.14^{b}

Means (n=3) with different letter in the column are significantly different (p<0.05).

Key: *WKB 1 = 100% wheat flour (**Negative control**); WKB 2 = 80% wheat flour + 20% kidney bean flour; WKB 3 = 60% wheat flour + 40% kidney bean flour; WKB 4 = 40% wheat flour + 60% kidney bean flour*

Table 3. Amino acid profiles of cookies samples (%)

Samples/ Amino acids	WKB 1	WKB 2	WKB 3	WKB 4	Average	±Std	#LSD (p<0.05)
Aspartic acid	7.27	7.62	12.87	13.13	11.21	1.11	0.34
Threonine	2.73	2.53	2.92	3.23	2.89	0.35	0.04
Serine	3.56	4.34	4.44	4.85	4.54	0.27	0.34
Glutamic acid	9.96	10.52	12.01	17.36	13.30	1.60	1.75
Proline	2.32	2.10	2.78	2.04	2.31	0.41	0.51
Glycine	3.16	3.18	3.21	3.28	3.22	0.05	0.74
Alanine	2.73	2.58	2.52	3.18	2.76	0.36	0.03
Cystine	1.43	1.22	0.39	1.51	1.04	0.58	0.02

Samples/ Amino acids	WKB 1	WKB 2	WKB 3	WKB 4	Average	±Std	#LSD (p<0.05)
Valine	4.11	4.28	4.76	4.64	4.56	0.25	0.03
Methionine	2.18	2.14	2.62	2.91	2.56	0.39	0.10
Isoleucine	2.59	2.67	2.70	2.69	2.69	0.02	0.61
Leucine	4.84	3.48	3.91	4.62	4.00	0.58	0.21
Tyrosine	3.41	3.03	3.49	4.31	3.61	0.65	0.24
Phenylalanine	2.28	4.32	4.42	7.98	5.57	0.08	0.11
Histidine	3.22	3.4	3.72	4.13	3.75	0.37	0.12
Lysine	3.78	3.12	3.7	4.72	3.85	0.81	0.03
Arginine	2.54	2.61	2.88	3.61	3.03	0.52	0.04
Tryptophan	0.87	1.49	0.91	1.32	1.24	0.30	0.01
TEAA	29.34	33.07	48.04	44.16	37.75	3.77	1.11
TNEAA	30.89	31.56	38.22	45.35	38.38	6.90	1.12
TAA	60.18	64.63	86.26	89.51	76.13	4.55	1.03
HAA	20.26	27.31	28.5	34.20	30.00	3.68	0.04
BV (%)	70.38	72.47	83.22	86.28	80.66	4.25	1.01

TEAA- Total Essential amino acids, TNEAA- Total Non-essential amino acid, TAA- Total amino acid., #LSD = Least significant difference. Key: WKB 1 = 100% wheat flour (Negative control); WKB 2 = 80% wheat flour + 20% kidney bean flour; WKB 3 = 60% wheat flour + 40% kidney bean flour; WKB 4 = 40% wheat flour + 60% kidney bean flour

Table 4. Physical properties of different cookies samples

Samples/ Parameters	WKB 1	WKB 2	WKB 3	WKB 4	Commercial sample
Weight (g)	5.20 ± 0.07^{e}	6.26 ± 0.02^{c}	6.25 ± 0.02^{a}	6.41 ± 0.01^{d}	6.51 ± 0.01^{ab}
Width (mm)	45.70 ± 0.17^{a}	45.82 ± 0.06^{a}	45.88 ± 0.46^{a}	45.72 ± 0.14^{a}	45.03 ± 0.58^{a}
Thickness (mm)	5.03 ± 0.08^{a}	5.06 ± 0.28^{a}	5.03 ± 0.51^{a}	5.03 ± 0.43^{a}	5.10 ± 0.01^{ab}
Spread ratio	11.96 ± 0.04^{b}	11.89 ± 0.01^{b}	12.22 ± 0.01^{a}	12.26 ± 0.02^{a}	8.83 ± 0.04^{c}

Means (n=3) with different letter in the row are significantly different (p<0.05).

Key: *WKB 1 = 100% wheat flour; WKB 2 = 80% wheat flour + 20% kidney bean flour; WKB 3 = 60% wheat flour + 40% kidney bean flour; WKB 4 = 40% wheat flour + 60% kidney bean flour*

Table 5. Sensory attributes of different cookies samples

SAMPLES	APPEARANCE	TASTE	TEXTURE	MOUTHFEEL	CRUMBLINGS	AROMA	OVERRALL ACCEPT.
WKB 1	8.20^{b}	7.70^{b}	7.45^{b}	7.90^{b}	7.75^{b}	7.50^{b}	8.20^{b}
WKB 2	7.05^{c}	6.85^{c}	7.45^{b}	7.55^{c}	7.40^{c}	6.65^{c}	6.95^{c}
WKB 3	7.30^{c}	6.25^{cd}	6.75^{d}	6.90^{d}	6.85^{d}	6.55^{c}	6.50^{d}
WKB 4	7.45^{c}	6.70^{c}	6.90^{cd}	6.10^{d}	6.50^{d}	6.70^{c}	6.85^{cd}
Commercial product	8.90^{a}	8.80^{a}	8.85^{a}	8.90^{a}	8.65^{a}	8.70^{a}	9.00^{a}

Means (n=50) with different letter in the column are significantly different (p<0.05).

Key: *WKB 1 = 100% wheat flour; WKB 2 = 80% wheat flour + 20% kidney bean flour; WKB 3 = 60% wheat flour + 40% kidney bean flour; WKB 4 = 40% wheat flour + 60% kidney bean flour*

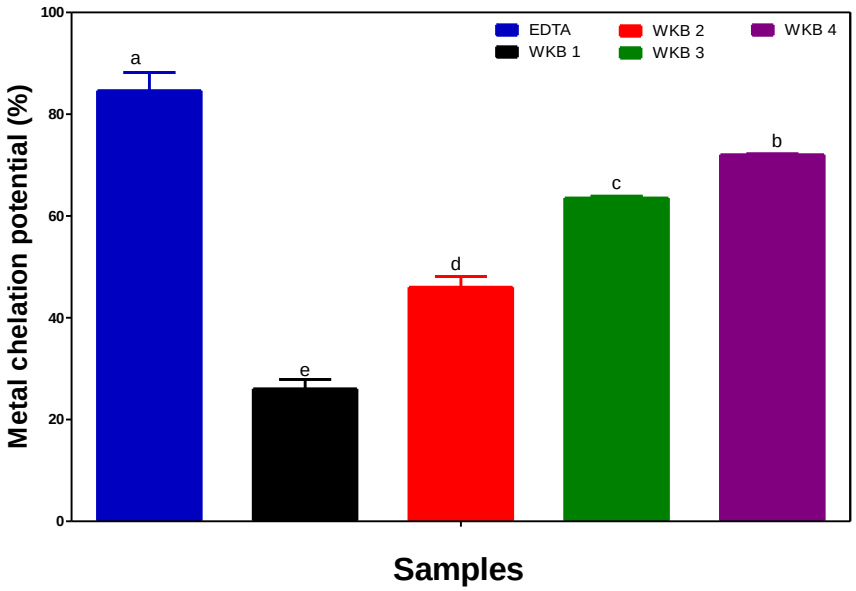

Fig. 3. Metal chelation potential of different cookies samples

bars (n=3) with different letter are significantly different (p<0.05), Key: WKB 1 = 100% wheat flour (Negative control); WKB 2 = 80% wheat flour + 20% kidney bean flour; WKB 3 = 60% wheat flour + 40% kidney bean; WKB 4 = 40% wheat flour + 60% kidney bean

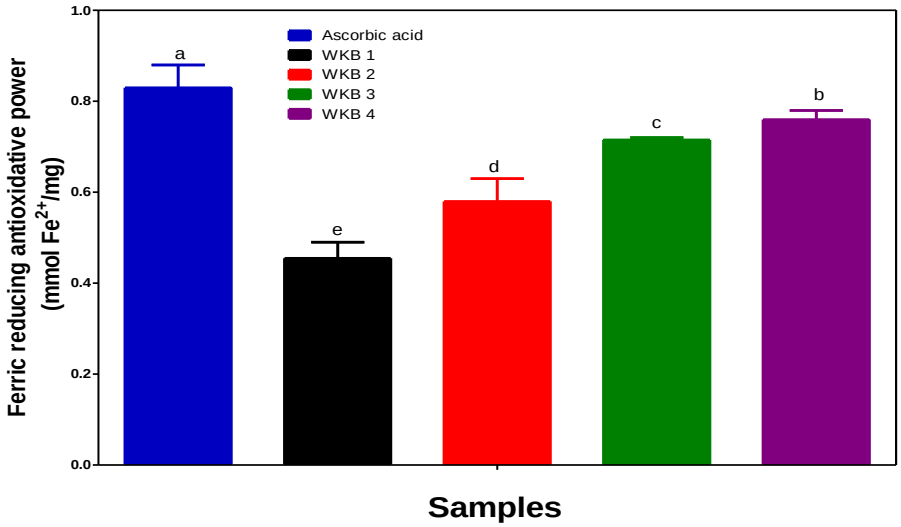

Fig. 4. Ferric reducing properties of different cookies samples

Bars (n=3) with different letter are significantly different (p<0.05), Key: WKB 1 = 100% wheat flour (Negative control); WKB 2 = 80% wheat flour + 20% kidney bean flour; WKB 3 = 60% wheat flour + 40% kidney bean; WKB 4 = 40% wheat flour + 60% kidney bean

3.5 Physical Properties of the Cookies

The physical properties of the cookies are presented in Table 4. The weights of the experimental cookies samples ranged from 5.20 to 6.41 g when compared to the commercial (control) sample that weighed about 6.51 g. The result of the physical properties is showing that the fortification process, which involved addition of kidney bean flour into the wheat flour is not adding unnecessary weights to the experimental composite cookies. However, the width of the cookie samples ranged from 45.70 mm (sample WKB1) to 45.88 mm (sample WKB3) when compared to 45.03 mm obtained for the commercial (control) sample. Meanwhile, the

thickness of the cookie samples ranged from 5.03 to 5.06 mm with samples WKB1, WKB3 and WKB4 having the same values (5.03 mm), respectively, when compared to 5.10 mm obtained for the commercial (control) sample. Hence, the spread ratio obtained for the cookies samples ranged from 11.89 to 12.26 as against 8.83 of the commercial (control) sample (Table 4). It can be concluded that sample WKB4 prepared from 40% wheat flour and 60% kidney bean flour possessed the best physical properties with better thickness and weight. The present observation on this blend is similar to the previous findings on cookies from unripe plantain-crayfish-wheat composite flour as reported [15].

3.6 Sensory Attributes of the Cookies

The sensory qualities of the cookie samples produced from the wheat-kidney bean flour blends are presented in Table 5. The result revealed that the addition of the kidney bean flour to the wheat flour during cookies production slightly affected the appearance of the composite cookies as shown in the low ratings observed in sample WKB2. The ratings of the appearances of the composite cookies (WKB2, WKB3 and WKB4) ranged between 7.05 to 7.45 and were not significantly (*P>0.*05) different each other, although these samples were significantly (P<0.05) different from the commercial product (8.90). The tastes of the samples were rated from 6.25 to 7.70, with the sample WKB1 and commercial sample (negative and positive controls, respectively) having the significant (P<0.05) better taste than the other composite experimental samples. This is due to their production from the well-known wheat flour as well as their commonality among the consumers, that is, most people are already accustomed to these products. Notably, taste, a sensation perceived by the tongue included the sweetness, saltiness, sourness and bitterness nature of any food product. Moreso, the high percentage of kidney bean flour, which is not as smooth as wheat flour in sample WKB4, might have affected its texture when compared to other cookies samples and as such, was rated low in mouth-feel when compared to the cookies samples made from the composite flour blends. However, the crumbling ratings of the samples slightly favoured the samples WKB2 and WKB3 while the high amount of the kidney beans in both WKB3 and WKB4 slightly affected their perceived aroma by the panelists, when compared to the control sample WKB1. Overall, all the experimental samples were acceptable by the panelists as shown in the close-range ratings obtained in the present study. This observation was also reported for the cookie samples made from wheat-unripe plantain-crayfish composite flour [15].

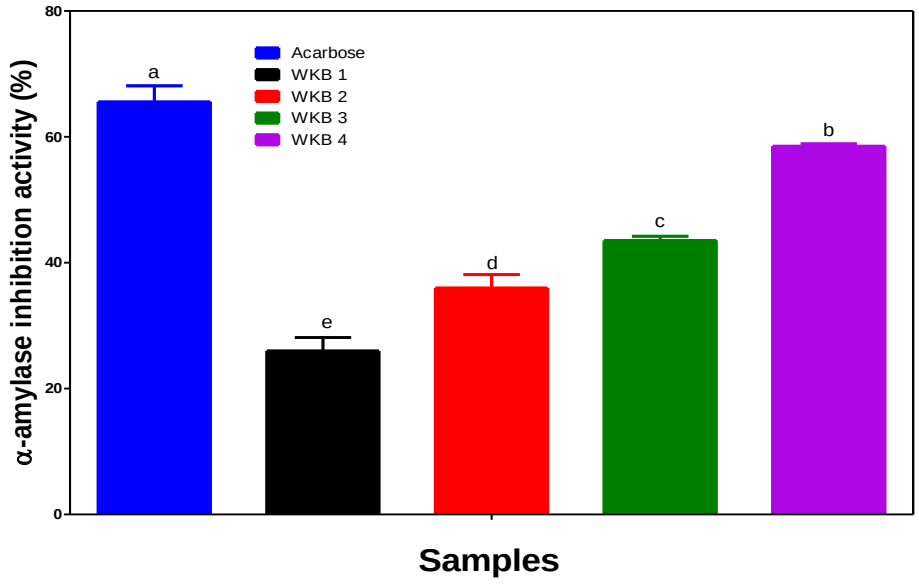

Fig. 5. *In vitro* α-amylase inhibition activities of different cookies samples

Bars (n=3) with different letter are significantly different (p<0.05).

Key: WKB 1 = 100% wheat flour (Negative control); WKB 2 = 80% wheat flour + 20% kidney bean flour; WKB 3 = 60% wheat flour + 40% kidney bean; WKB 4 = 40% wheat flour + 60% kidney bean

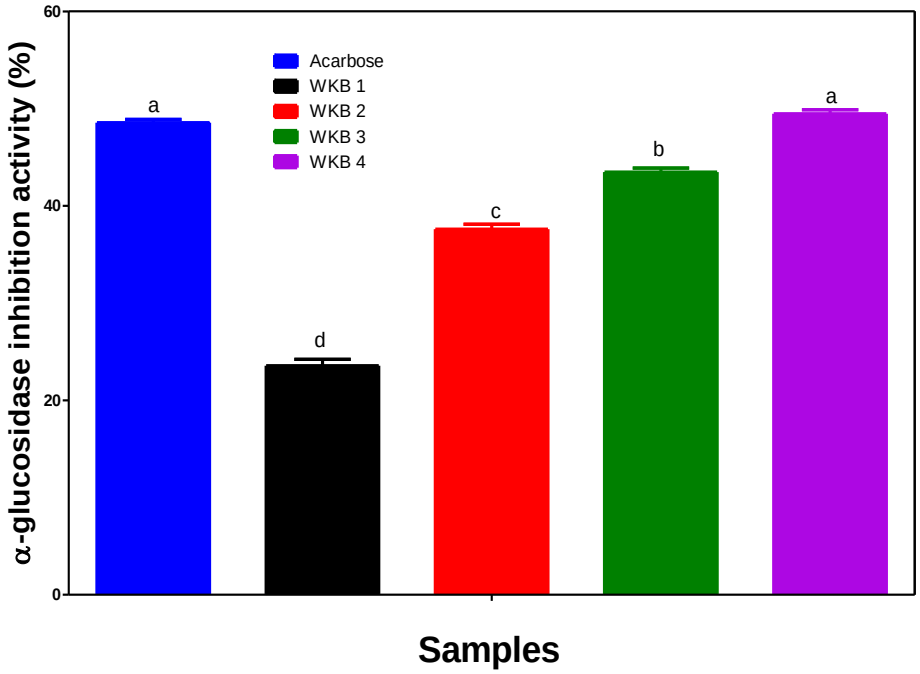

Fig. 6. *In vitro* α-glucosidase inhibition activities of different cookies samples

Bars (n=3) with different letter are significantly different (p<0.05), Key: WKB 1 = 100% wheat flour (Negative control); WKB 2 = 80% wheat flour + 20% kidney bean flour; WKB 3 = 60% wheat flour + 40% kidney bean; WKB 4 = 40% wheat flour + 60% kidney bean

4. CONCLUSION

From the result of this study, the cookie sample from 40% wheat flour and 60% kidney bean flour gave the best nutritional properties in terms of proximate composition, amino acid composition and antioxidants and anti-diabetic (inhibition of α-amylase and α-glucosidase) properties. It has high value of hydrophobic amino acid which may be advantageous to the management of type 2 diabetes mellitus.

The study also established that the *in vitro* antioxidant activities of the cookie sample from 40% wheat flour and 60% kidney bean flour were found to be comparable to ascorbic acid activities. The study further established that the cookie sample from 40% wheat flour and 60% kidney bean flour has high inhibitory effects against α- amylase and α- glucosidase activities and moreso, found comparable to acarbose, a known anti-diabetic drug. Hence, its preparation might not have negative effect on its application as functional diet to modulate type 2 diabetes mellitus. It can therefore be used in the management of chronic diseases instead of the use of synthetic products, that has had long histories of negative side effects.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Olugbuyi AO, Oladipo GO, Malomo SA, Ijarotimi SO, Fagbemi TN. Biochemical ameliorating potential of optimized dough meal from plantain (Musa AAB), soycake (*Glycine max*) and rice bran (*Oryza sativa*) flour blends in Streptozotocin-induced diabetic rats. Applied Food Research. 2022;1-11. Available:https://Doi.Org/10.1016/J.Afres.2022.100097
2. Oluwajuyitan TD, Ijarotimi OS. Nutritional, antioxidant, glycaemic index and antihyperglycaemic properties of improved traditional plantain-based (*Musa abb*), dough meal enriched with tigernut (*Cyperus esculentus*) and defatted soybeans (*Glycine max*) cake for diabetics patients. Heliyon. 2019;e1504-9.
3. Arise KA, Malomo SA, Owolabi O, Arise OR. Proximate, antioxidant and sensory properties of Tidbit snacks from cassava

enriched with Benniseeds. ACS Food Science and Technology. 2021;1(2):268–274.
Available:https://dx.doi.org/10.1021/acsfoodscitech.0c00054

4. Siddeeg A, Xu Y, Jiang Q, Xia W. *In vitro* antioxidant activity of protein fractions extracted from seinat (*Cucumis melo* var. tibish) seeds. CyTA – Journal of Food. 2015;13(3):472-481
5. Ou S, Kwok KC, Li Y, Fu L. *In vitro* study of possible role of dietary fiber in lowering postprandial serum glucose. Journal of Agricultural and Food Chemistry. 2001;49 (2):1026-1029.
6. Thammarat K, Leena N, Punnanee S, Soottawat B. Functional and antioxidative properties of Bambara groundnut (*Voandzeia subterranea*) protein hydrolysates. International Food Research Journal. 2015;22(4):1584–1595
7. Mundi S, Aluko R. Physicochemical and functional properties of kidney bean albumin and globulin protein fractions. Food Research International. 2012;48 (1):299-306.
8. Akinyede AI, Malomo SA, Fagbemi TN, Osundahunsi OF, Aluko RE. Polypeptide profile, amino acid composition and some functional properties of calabash nutmeg (*Monodora myristica*) proteins. Journal of American Oil Chemists Society. 2017;94:1361-1371
9. Giasson BI, Duda JE, Murray IV, Chen Q, Souza JM. Oxidative damage linked to neurodegeneration by selective alpha-synuclein nitration in synucleinopathy lesion*s.* Science*.* 2002;3:985–989.
10. Sibian MS, Riar CS. Formulation and characterization of cookies prepared from the composite flour of germinated kidney bean, chickpea, and wheat. Legume Science. 2020;1–12.
Available:https://doi.org/10.1002/leg3.42
11. Bhatt MS, Rajinder KG. Bread (composite flour) formulation and study of its nutritive, phytochemical and functional properties. Journal of Pharmacognosy and Phytochemistry. 2015;4(2):254-268.
12. Ijarotimi OS, Keshinro OO. Protein quality, hematological properties and nutritional status of albino rats fed complementary foods with fermented popcorn, African locust bean, and Bambara groundnut flour blends. Nutrition Research and Practice. 2012;6(5):381-388.
13. AOAC, 2012. Association of Official Analytical Chemist, Official Methods of Analysis, 19th edition. AOAC International, Maryland, USA. 2012;92-156
14. Malomo SA, Udeh CC. Quality and *in vitro* estimated glycemic index of cookies from unripe plantain-crayfish-wheat composite flour. Applied Tropical Agriculture. 2018;23(2):82-89
15. Malomo SA, Nwachukwu ID, Girgih AT, Idowu AO, Aluko RE, Fagbemi TN. Antioxidant and renin-angiotensin system (RAS) inhibitory properties of cashew nut and fluted-pumpkin protein hydrolysates. Polish Journal of Food and Nutrition Science. 2020;70(3):275-289.
16. Mune-Mune MA, Minka SR, Mbome IL, Etoa FX. Nutritional potential of bambara bean protein concentrate. Pakistan Journal of Nutrition. 2011;10(2):112-119.
17. Sheikh JH, Tsujiyama MT, Md Ashabul I, Rajat SB, Hitoshi A. Total phenolic content, anti-oxidative, anti- amylase, anti-glucosidase and anti- histamine release activities of Bangladeshi fruits. Food Science Technological Research. 2008;14: 261-68.
18. Arise KA, Malomo AS, Abdulrasaq AA, Arise RO. Quality attributes and consumer acceptability of custard supplemented with Bambara groundnut protein isolates. Applied Food Research. 2022;2:1-6.
Available:https://doi.org/10.1016/j.afres.2022.100056
19. Food and Agricultural Organization (FAO). FAOSTAT. Food and Agricultural Organization of the United Nations. 2014; 240.
20. Pereira MA, Jacobs DR, Pins JJ, Raatz SK, Gross MD, Slavin JL, Seaquist ER. Effect of whole grains and insulin sensitivity in overweight hyperinsulinemic adults. American Journal of Clinical Nutrition. 2014;75:848– 855.
21. Udeh C, Ifie I, Job A, Malomo SA. Kidney bean protein products as potential antioxidative and antihypertensive alternatives for non-pharmacological inhibition of angiotensin-converting enzymes. Journal of Scientific African. 2021;11(e00693):1-16.
Available:https://doi.org/10.1016/j.sciaf.2021.e00693.
22. Olugbuyi AO, Malomo SA, Ijarotimi SO, Fagbemi TN. Amino acids profile, glycaemic index/load, *in vitro* antioxidant and sensory attributes of instant plantain-

based dough meal enriched with soycake and rice bran flour blends. Journal of Culinary Science. 2021;1-24. Available:https://doi.org/10.1080/15428052.2021.2016530

23. Wu J, Aluko RE, Nakai S. Structural requirements of Angiotensin I-Converting Enzyme inhibitory peptides: Quantitative structure-activity relationship modeling of peptides containing 4-10 amino acid residues. QSAR Combinational Science. 2006;25(10):873–880
24. Malomo SA, Aluko RE. A comparative study of the structural and functional properties of isolated hemp seed (*Cannabis sativa* L.) albumin and globulin fractions. Food Hydrocolloids. 2015;43: 743-752
25. Olagunju AI, Oluwajuyitan TD, Oyeleye SI. Effect of plantain bulb's extract-beverage blend on blood glucose levels, antioxidant status, and carbohydrate hydrolysing enzymes in streptozotocin-induced diabetic rats. Preventive Nutrition and Food Science. 2020;25(4):362–374. Available:https://doi.org/10.3746/pnf.2020.25.4.362
26. Zhang S, Li X, Luo H, Fang ZZ, Ai H. Role of aromatic amino acids in pathogeneses of diabetic nephropathy in Chinese patients with type 2 diabetes. Journal of Diabetes and its Complications. 2020;107667.
27. Adefegha SA, Oboh G, Adefegha MI, Henle T. Alligator pepper/grain of paradise(*Aframomum melegueta*) modulates Angiotensin-I converting enzyme activity, lipid profile and oxidative imbalances in a rat model of hypercholesterolemia, Pathophysiology. 2016;23:191–202.
28. Odebode FD, Ekeleme OT, Ijarotimi OS, Malomo SA, Idowu AO, Badejo AA, Fagbemi TN. Nutritional composition, antidiabetic and antilipidemic potentials of flour blends made from unripe plantain, soybean cake, and rice bran. Journal of Food Biochemistry. 2017; 42(4):e12447.
29. Oluwajuyitan TD, Ijarotimi OS, Fagbemi TN. Nutritional, biochemical and organoleptic properties of high protein-fibre functional foods developed from plantain, defatted soybean, rice-bran and oat-bran flour. Nutrition and Food Science. 2020;51(6):1-30.
30. Kazeem MI, Adamson JO, Ogunwande IA. Modes of inhibition of α-amylase and α-glucosidase by aqueous extract of MorindalucidaBenth leaf. BioMed Research International. 2013;4:24-32.
31. Rege AA., Chowdhary AS. Evaluation of alpha-amylase and alpha-glucosidase inhibitory activities of *Rhizophora mucronata*. International Journal of Pharmaceutical Sciences and Research. 2014;5(6):2261.
32. Fuentes-Zaragoza E, riquelme-Navarrete MJ, Sánchez-Zapata E, Pérez-Alvarez JA Resistant starch as functional ingredient: a review. Food Research International. 2010;43:931–942
33. Agu KC, Eluehike N, Ofeimun RO, Abile D, Ideho G, Ogedengbe MO, Elekofehinti OO. Possible anti-diabetic potentials of Annona muricata (soursop): inhibition of α-amylase and α-glucosidase activities. Clinical Phytoscience. 2019;5(1):21.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/115687