Original Research Article

Anti-diabetic potentials of cookies from wheat, sweet potato and African yam beans (*Sphenostylisstenocarpa*) composite flour blends

ABSTRACT

Cookies offered several important advantages including the wide consumption, relatively long shelf life, good eating quality, highly palatable and acceptable in most countries. This study thus, investigated the antioxidant and anti-diabetic properties of cookies produced from wheat-African yam bean-sweet potato composite flour blends at different ratios. The African yam bean and sweet potato were thoroughly processed, milled, sieved and mixed with the commercial wheat flour. Four blends were prepared by using the trial mixing ratio and 100% wheat flour and later used to bake cookies. The proximate, amino acid compositions, antioxidant, amylase and glucosidase inhibitory activities as well as physical properties of the composite cookies were examined while panelists were assigned to assess the cookie samples. The results of the proximate composition showed that the composite flour technology had significant ($p<0.05$) positive effects on the cookies. Meanwhile, the amino acid compositions of the cookies revealed that the cookie samples were of high aromatic and hydrophobic amino acids, respectively with optimum physical properties. No panelist showed a total dislike for the taste of any of the samples. The added soybean seedsflours showed no significant ($p<0.05$) effect on the acceptability and preference of the samples. Hence, it is possible to produce better protein and high nutritional cookies from these flour blends. We therefore concluded that the cookies rich in antioxidants and anti-diabetic potentials could be produced from wheat-African yam bean-sweet potato composite flours

Keywords: African yam bean, inhibition, amino acid, amylase, glucosidase, cookies

1. INTRODUCTION

African yam bean (*Phaseolus vulgaris* L) are common legumes consumed worldwide as excellent plant-based source of protein [1].Endogenous protein displayed anti-inflammatory properties, as part of the immune system, the complement system which is made up of 30 plasma proteins plays an important role in fighting pathogen by interacting closely with other components of the immune system [1]. It helped anti-bodies and phagocyte to eliminate pathogen.African yam beans are good source of proteins and trace mineral, which is essential co-factors in a number of enzymes important in energy production and anti-oxidant defenses, which disarmed free radicals produce within the mitochondria [2].Therefore, these beans may aid weight loss, promote colon health, and moderate blood sugar levels [2]. Sweet potato is an important crop in many parts of the world, which is classified as a storage root rather than a tuber. The storage roots of sweet potato serve as staple food, animal feed [3], and to a limited extent as a raw material for industrial purposes as a starch source and for alcohol production [3]. In Japan dehydrated sweet potato is ground into flour, which is cooked for human consumption. Sweet potato starch is used for the manufacture of adhesives, textile and paper sizing and in the confectionery and baking industries. In most parts of the tropics, sweet potato is consumed

boiled, baked, roasted or fried. Preparation practices vary according to the location. Sweet potato can also be used as a raw material for the feed industry [3]. Sweet potato can be processed into different products for value addition. The most promising ones are dried chips, starch and flour [4]. Sweet potato flour can be a source of natural sweetener, dietary fibre and energy in bakery goods and beverages and add flavour and colour to many other processed products [4].

A current trend in nutrition is the consumption of low-carbohydrate diets, including slowly digested food products, as well as an increased intake of functional foods [5]. The food professionals/industries are faced with the challenge of producing food products containing functional ingredients in order to meet the nutritional requirements of individuals with health challenges. Adequate nutrition, which is achieved through consumption of a balanced healthy diet, is a fundamental pillar of human life, health and development across the entire life spam [6]. Therefore, cookies can serve as vehicle for delivery of important nutrients if made readily available to the population [7]. Cookies are small flat, baked confectionary which is either crisp or soft but firm [7]. It is majorly produced from wheat flour and other ingredients. Cookies are consumed extensively all over the world as a snack food and on a large scale in developing countries where proteinand caloric malnutrition are prevalent [4]. Research has shown that cookies can also be produced from other composite flour that are of more health benefits than the regular wheat flour[8].Currently, there is a great awareness among the consumers in the preventive health care with respect to the development of natural functional foods ingredients from plant materials due to their high bio-digestion rate with no residual or negative side effects that were known with series of synthetic drugs. Hence, the production of cookies from the flour blends obtained from these well-known crops (sweet potato and African yam bean) would serve as basic functional foods aiming to be used in the management of the chronic cardiovascular diseases, such as diabetes, etc. Thus, the main objective of this study is to examine the *in vitro* anti-diabetic potentials of cookies from sweet potato, African yam bean and wheatcomposite flours.This is done by specifically determining the proximate, amino acid compositions, antioxidant, amylase and glucosidase inhibitory activities as well as physical and organoleptic properties of the composite cookies.

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 Ado Ekiti, Nigeria. The sweet poatato and African yam bean seeds were obtained from the King's market, Ondo, 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 African yam bean flour

The African yam bean flour was obtained according to the method previously described [9]. The seeds were sorted to remove foreign materials, defected seeds, insects etc. The healthy beans were washed and conditioned for germination by soaking overnight, drained, spread on flat trays and covered in a closed environment (20-25 ^{o}C) for about 2-3 days for sprouting. The

germinated beans were dried to remove the sprouts, dehulled, milled in attrition mill and finally sieved through 0.4 mm wire mesh to obtain the final flour for further analysis.

2.3 Preparation of Sweet Potato flour

Sweet potato tubers were peeled and cut into thin pieces manually. Firstly, the potato slices were immersed in a solution containing 1% NaCl, 1% potassium metabisulphite and 0.5% citric acid for 30 min to prevent browning reactions and enhance the colour of the flour. The sweet potato slices were dried on perforated trays in a tray dryer at 55^0C till constant weight achieved. The dried sweet potato chips were milled into flour using the laboratory grinder and passed through 0.25μm mesh sieve, packed in airtight containers and stored till further use according to the previously described method [4].

2.3 Formulation of composite flour blends

The composite flour blends were formulated from wheat, sweet potato and African yam bean flour blends as stipulated in the Table 1 *viz:*

Table 1- Flour blends formulations

Samples	Formulations	Wheat	Sweet potato	African yam beans	Total (%)
WSA 1	Wheat (commercial flour)	100	0	0	100
WSA 2	Wheat+ Sweet potato + African yam bean flour	80	10	10	100
WSA 3	Wheat + Sweet potato + African yam bean flour	70	20	10	100
WSA 4	Wheat + Sweet potato + African yam bean flour	60	20	20	100

2.5 Production of cookies

Cookies were produced as previously described [4] 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.

2.6 Proximate composition analysis

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 by Association of Official Analytical Chemist[10]. 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.

2.7 Amino acid analysis

The amino acid profiles of the cookies were determined using the High-performance liquid chromatography (HPLC) method as previously described [11].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.

2.8 Determination of DPPH radical scavenging activity

The radical scavenging activities of the flour and dough samples were determined using the stable radical DPPH (2,2-diphenyl-1-picrylhydrazyl hydrate) as described by [11]. 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 517nm. To 1ml of different concentrations of the extract or standard (ascorbic acid) in a test tube was added 1ml of 0.3mM DPPH in methanol. The mixture was mixed and incubated in the dark for 30min after which the absorbance was read at 517nm against a DPPH control containing only 1ml methanol in place of the extract.

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

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

2.9 Determination of ferric reducing antioxidant power (FRAP)

Ferric reducing or antioxidant power was determined as described [11]. 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.

2.10 Determination of hydroxyl radical scavenging activity

The deoxyribose assay was used to determine the hydroxyl radical scavenging activity in an aqueous medium according to the procedures previously described [11]. 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.

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

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

2.11 Determination of iron chelating activity

Metal chelating activity was measured as described [11]. 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.

2.12 Inhibition of α-amylase activity

The *in-vitro* α-amylase activity can be measured by hydrolysis of starch in presence of α-amylase enzyme. This process was quantified by using iodine, which gives blue colour with starch. The reduced intensity of blue colour indicates the enzyme-induced hydrolysis of starch in to monosaccharides. If the substance possesses α-amylase inhibitory activity, the intensity of blue colour will be more. In other words, the intensity of blue colour in test sample is directly proportional to α-amylase inhibitory activity [6].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).

2.13 Inhibition of α-glucosidase activity

The α-glucosidase inhibition activity was performed according to the slightly modified method [6]. 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 37 ^oC 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 37^oC 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.

2.14 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 [7]. 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.

2.15 Evaluation of sensory attributes

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 [7].

2.16 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 cookies

The crude protein contents of the cookies presented in Table 2 showed the ranges of results from 15.16 to 28.15%, which is higher than the 4.50 and 8.90% reported for wheat-potato flour blends-produced biscuit [12]. The increase in the protein level of cookies from the wheat, African yam bean (AYB) and sweet potato flour blends (WAS 1-5) could possibly come from AYB fraction of the blended flour that is rich in protein. The increase in protein value of the cookies indicated high nutritional value. The consumption of 17-25 g of soy protein per day had been reported to reduce serum cholesterol to 9.3% on the average, while the low-density lipoprotein cholesterol (LDL) reduced to about 13% [13].

The crude fiber ranged between 1.85 and 4.10%. The result is higher than the 0.37 and 1.48% reported for wheat-acha-soybean composite cookies [14]. The higher fiber content could have come from the sweet potato fraction of the flour since AYB used was de-hulled. The increase in fiber of the cookies as a result of supplementation agreed with the past findings [4]. Crude fibre helped in the prevention of heart diseases, colon cancer, diabetes etc. Wheat flour would not be a better source of fibre content since it had significantly lower crude fibre content.

Therefore, it will be useful if sweet potato is added to it and used in food formulation to help relieve constipation.

The crude fat in the cookies ranged between 5.48 and 7.61%, with highest concentration of fat (7.61%) observed in cookies produced from sample WAS 1, which is lower than the 10.97 - 18.93% previously reported for wheat-potato biscuit [12]. However, cookies with reasonable concentrations of fat were produced from all the composite flour ratios.

Table 2- Proximate compositions of composite cookies **(%)** (on dry weight basis)

Samples	Moisture	Crude protein	Crude fiber	Crude fat	Crude ash	Carbohydrate
WAS 1	8.13±0.13^{b}	15.16±0.27^{e}	2.67±0.02^{b}	7.61±1.22^{b}	2.61±0.21^{a}	66.95±1.26^{a}
WAS 2	8.67±0.18^{a}	28.15±0.33^{a}	2.88±0.01^{b}	5.48±0.57^{a}	2.28±0.20^{b}	54.21±1.01^{ab}
WAS 3	8.33±0.12^{b}	22.59±0.12^{b}	2.05±0.03^{b}	6.28±0.56^{c}	2.60±0.19^{ab}	60.49±0.80^{c}
WAS 4	8.31±0.25^{b}	19.52±0.51^{c}	4.10±0.02^{a}	6.31±0.64^{ab}	2.26±0.20^{bc}	60.81±0.66^{bc}
WAS 5	8.20±0.12^{b}	16.58±0.04^{d}	1.85±0.03^{c}	6.12±0.47^{b}	2.13±0.20^{bc}	67.19±0.64^{b}

Means (n=3) with different letter in the column are significantly different ($p<0.05$).
Key:WAS 1 = 100% wheat (commercial flour); WAS 2 = 45% wheat flour + 20% sweet potato flour + 35% African yam bean flour; WAS 3 = 40% wheat flour + 30% sweet potato flour + 30% African yam bean flour; WAS 4 = 35% wheat flour + 40% sweet potato flour + 25% African yam bean flour; WAS 5 = 30% wheat flour + 50% sweet potato flour + 20% African yam bean flour

The crude ash contents ranged between 2.13 and 2.61%, with the highest value observed in cookies produced from sample WAS 1. The ash content of food material could be used as an index of mineral constituents of the food because ash is the inorganic residue remaining after the water and organic matter have been removed by heating in the presence of an oxidizing agent [15]. The carbohydrate values ranged between 54.21 and 67.19%, which was higher than the 42.22 to 50.45% previously reported for breadfruit-breadnut-wheat composite bread [16]. This observation may be attributed to the high content of carbohydrate in sweet potato than AYB and wheat hence, the higher carbohydrate content in the cookies, made it a quick source of metabolizable energy and thus, to be assisted in fat metabolism.

3.2 Amino acids profiles of cookies samples

The results of the amino acids profile of the cookies made from WAS 1 (control), 2, 3, 4 and 5 composite flours were presented in Table 3 with observable significant ($p<0.05$) differences. The result showed that eighteen (18) different amino acids were determined including the nine (9) essential amino acid *viz:* phenylalanine, isoleucine, valine, threonine, leucine, isoleucine, lysine, histidine, methionine. Further observation of the result (Table 3) revealed that the lysine and aspartic acid contents were higher in the composite cookies (WAS 2, 3, 4 and 5) when compared to the control sample (WAS 1). Contrarily, cookie sample WAS 2 has higher amounts of glutamic acid, arginine (a precursor of nitric oxide (good visodilator), proline, cystine and histidine than in cookie samples WAS 4 and 5, respectively. Notably, the well-known aromatic amino acids such as tryptophan, tyrosine and phenylalanine (Table 3) were

all significantly ($p<0.05$) higher in sample WAS 2 than in other samples WAS 1, 3, 4 and 5, respectively. This could invariably have a positive contributory effect on its bioactivities and antioxidative effects[4, 6, 9, 11].

Table 3 – Amino acid compositions of cookies **(%)**

Samples/AA	WAS 1	WAS 2	WAS 3	WAS 4	WAS 5	Average	± Standard deviation
Aspartic acid	7.62	12.87	10.58	9.27	9.16	9.90	1.11
Threonine	2.53	3.92	3.59	2.23	2.73	3.00	0.35
Serine	4.34	4.44	3.23	4.85	3.56	4.08	0.27
Glutamic acid	8.52	14.01	12.49	11.36	11.27	11.53	1.60
Proline	2.10	2.78	2.26	2.04	2.19	2.27	0.41
Glycine	3.18	3.21	2.27	2.28	2.22	2.63	0.05
Alanine	2.58	3.52	2.89	2.18	2.58	2.75	0.36
Cystine	0.22	1.39	0.95	0.51	0.31	0.68	0.58
Valine	4.28	4.76	4.53	3.64	3.11	4.06	0.25
Methionine	2.14	2.62	2.45	2.91	2.14	2.45	0.39
Isoleucine	2.67	3.70	3.75	2.69	2.67	3.10	0.02
Leucine	3.48	3.91	2.90	2.62	2.44	3.07	0.58
Tyrosine	3.03	3.49	2.74	2.31	2.03	2.72	0.65
Phenylalanine	2.32	7.42	6.58	2.98	2.32	4.32	0.08
Histidine	3.02	5.72	3.39	3.13	3.31	3.71	0.37
Lysine	3.03	4.70	3.77	3.72	3.68	3.78	0.81
Arginine	2.31	3.88	3.42	2.61	2.54	2.95	0.52
Tryptophan	0.80	1.91	0.76	0.82	0.87	1.03	0.30

Key:WAS 1 = 100% wheat (commercial flour); WAS 2 = 45% wheat flour + 20% sweet potato flour + 35% African yam bean flour; WAS 3 = 40% wheat flour + 30% sweet potato flour + 30% African yam bean flour; WAS 4 = 35% wheat flour + 40% sweet potato flour + 25% African yam bean flour; WAS 5 = 30% wheat flour + 50% sweet potato flour + 20% African yam bean flour

3.3 Antioxidant properties of cookies

Past studies have demonstrated significant decrease of plasma antioxidants in the pathogenesis of diabetes and its associated complications, such as endothelial dysfunction and

atherosclerosis [17-18]. For instance, a low level of plasma antioxidants was more pronounced in elderly diabetic patients (Polidori *et al.*, 2001). Therefore, this brought a strong rationale for the therapeutic use of antioxidants in the treatment and prevention of diabetic complications. Although, phytochemicals (such as flavonoids, carotenoids, ascorbic acid and tocopherols) were the main sources of antioxidants [19] but other food crops, like snacks of high protein contents could be found exploitable as good antioxidants. This is because they have been shown to inhibit reactive oxygen species (ROS) production by inhibiting several ROS producing enzymes (namely; xanthine oxidase, cyclooxygenase, lipoxygenase, microsomal monooxygenase, glutathione-S-transferase, mitochondrial succinoxidase, NADH oxidase). Besides, they functioned in chelating trace metals and inhibiting phospholipases A2 and C [20].

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 22.89 to 73.89% when compared to the common and well-known antioxidant, ascorbic acid (78.89%). It was observed that the sample WAS 2 had the significant ($p<0.05$) highest property (~74%) when compared to sample WAS 1 (cookie from wheat flour only) that has ~23% activity.

The DPPH radical scavenging activity of the samples is obtained in the following ranges; 20.34-82.11% 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.

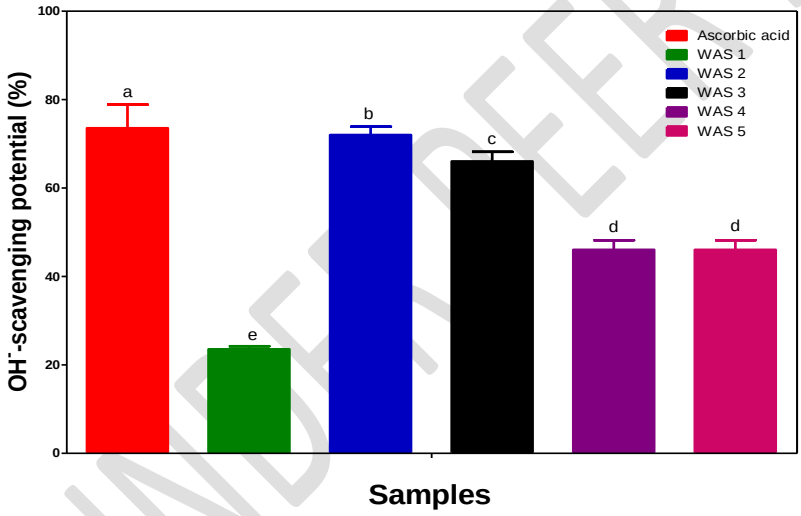

Fig 1: Hydroxyl (OH) radical scavenging potential of cookies
Bars (n=3) with different letter are significantly different ($p<0.05$).
Key:WAS 1 = 100% wheat (commercial flour); WAS 2 = 45% wheat flour + 20% sweet potato flour + 35% African yam bean flour; WAS 3 = 40% wheat flour + 30% sweet potato flour + 30% African yam bean flour; WAS 4 = 35% wheat flour + 40% sweet potato flour + 25% African yam bean flour; WAS 5 = 30% wheat flour + 50% sweet potato flour + 20% African yam bean flour

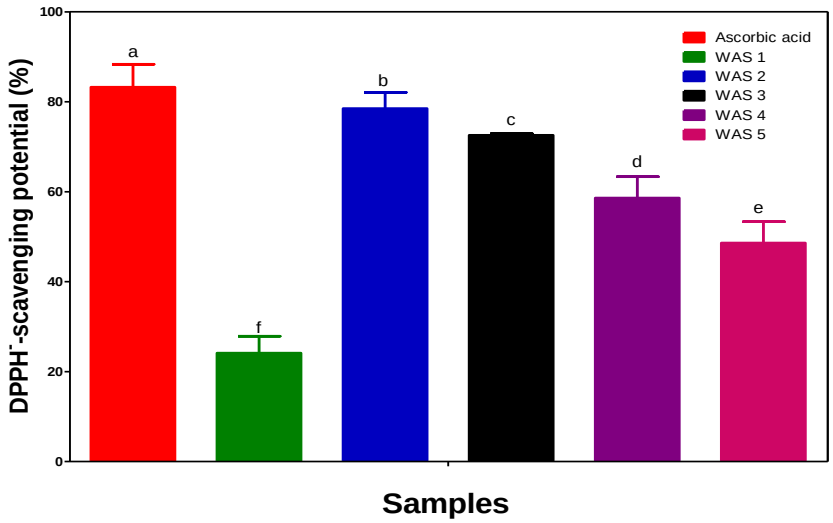

Fig 2: DPPH radical scavenging potential of cookies
Bars (n=3) with different letter are significantly different ($p<0.05$).
Key:WAS 1 = 100% wheat (commercial flour); WAS 2 = 45% wheat flour + 20% sweet potato flour + 35% African yam bean flour; WAS 3 = 40% wheat flour + 30% sweet potato flour + 30% African yam bean flour; WAS 4 = 35% wheat flour + 40% sweet potato flour + 25% African yam bean flour; WAS 5 = 30% wheat flour + 50% sweet potato flour + 20% African yam bean flour

A similar trend of low Fe^{2+} chelation antioxidant activity was observed in WAS 1 (24.22%) when compared to WAS 2 (72.20%) as presented in Fig 3. It was observed that the antioxidant activities of sample WAS 1, 3, 4 and 5 (with respect to hydroxyl, DPPH radical scavenging and Fe^{2+} chelation antioxidant activities) were significantly ($p<0.05$) lower than WAS 2. This implied that the samples WAS 1, 3, 4 and 5 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 WAS 2 also had highest ferric reducing antioxidative potentials (FRAP) when compared to others. For instance, WAS 2 and WAS 1 had 0.78 and 0.49 mmol Fe^{2+}/mg when compared with ascorbic acid (0.88 mmol Fe^{2+}/mg).

The relative higher antioxidant potentials 82.11%) of the cookie sample WKB4 may be attributed to its higher protein content (28.20%), glutamic acid (14.01%) and arginine amino acids (3.88%) 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 [21]. This finding also agreed with past study that reported on the contributions of antioxidants in diabetes and its complications [22]. Several studies have demonstrated significant decrease of cardiovascular disease such as diabetes and hypertension with consumption of foods that were rich in antioxidants [22, 23-24]. Hence, sample WAS 2 could potentially help in reducing the risk of chronic cardiovascular diseases.

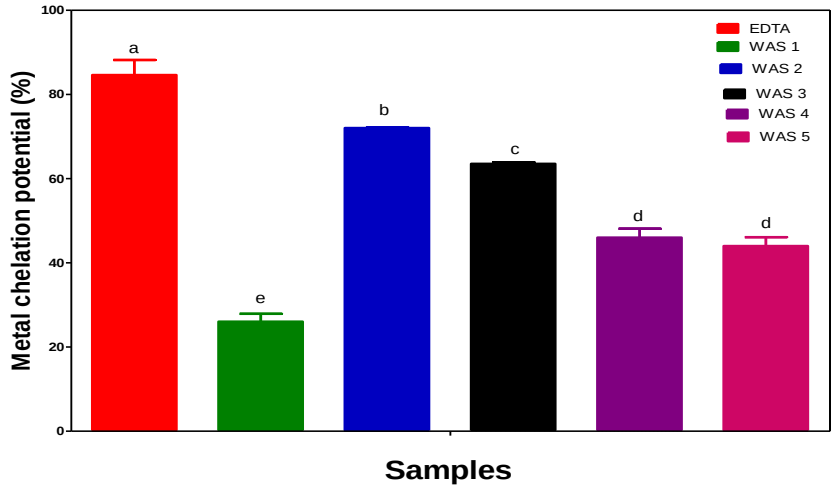

Fig 3: Metal chelation potential of cookies
Bars (n=3) with different letter are significantly different ($p<0.05$).
Key:WAS 1 = 100% wheat (commercial flour); WAS 2 = 45% wheat flour + 20% sweet potato flour + 35% African yam bean flour; WAS 3 = 40% wheat flour + 30% sweet potato flour + 30% African yam bean flour; WAS 4 = 35% wheat flour + 40% sweet potato flour + 25% African yam bean flour; WAS 5 = 30% wheat flour + 50% sweet potato flour + 20% African yam bean flour

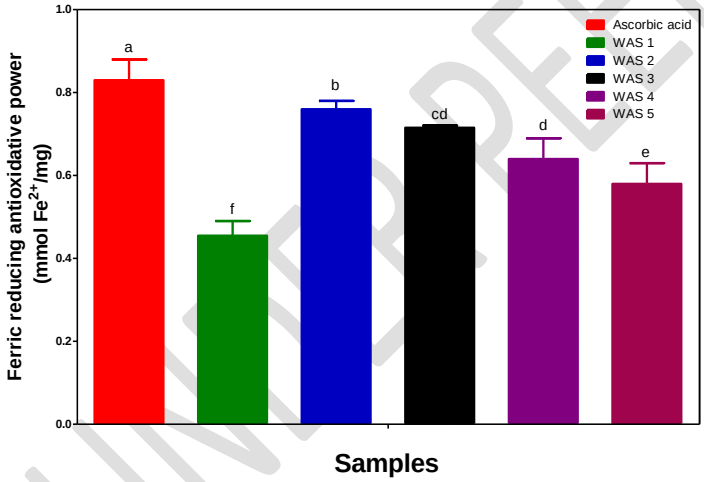

Fig 4: Ferric reducing properties of cookies
Bars (n=3) with different letter are significantly different ($p<0.05$).
Key:WAS 1 = 100% wheat (commercial flour); WAS 2 = 45% wheat flour + 20% sweet potato flour + 35% African yam bean flour; WAS 3 = 40% wheat flour + 30% sweet potato flour + 30% African yam bean flour; WAS 4 = 35% wheat flour + 40% sweet potato flour + 25% African yam bean flour; WAS 5 = 30% wheat flour + 50% sweet potato flour + 20% African yam bean flour

4.4 Anti-diabetic properties (α-amylase and α-glucosidase inhibition potentials) of cookies

A major strategy in diabetes management is the control of key enzymes linked with its metabolic pathways of diabetes. The inhibition of carbohydrate hydrolyzing enzymes such as α-amylase and α-glucosidase has been suggested to be one practical approach to the control of hyperglycemia in diabetic situations [25]. The carbohydrate hydrolyzing enzymes such as pancreatic α-amylase and intestinal α-glucosidase played major roles in carbohydrate hydrolysis and absorption [26]. Therefore, it is an effective approach to manage the blood glucose level via inhibiting the activities of the α-amylase and α-glucosidase enzymes [27].The data revealed in Fig 5 is the α-amylase inhibition abilities of the composite wheat, African yam bean and sweet potato (WAS) cookie samples. The percentage α –amylase inhibition of the cookie samples ranged from 28.11% (WAS 1) to 58.89% (WAS 2). The cookie WAS 2 had significant ($p<0.05$) higher α–amylase inhibition than the other samples (WAS 1, 3, 4 and 5) but lower than the activity obtained (68.12%) for acarbose, a common anti-diabetic drug. The higher proline, glutamic and aspartic amino acids obtained for WAS 2 (Table 3) might be accounted for its higher α–amylase inhibition [28].The highest α–amylase inhibition (58.89%) exhibited by WAS 2 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 24.22% (WAS 1) to 49.89% (WAS 2). The other composite cookie samples (WAS 1, 3, 4 and 5) have significant ($p<0.05$) less α- glucosidase inhibition than the WAS 2.

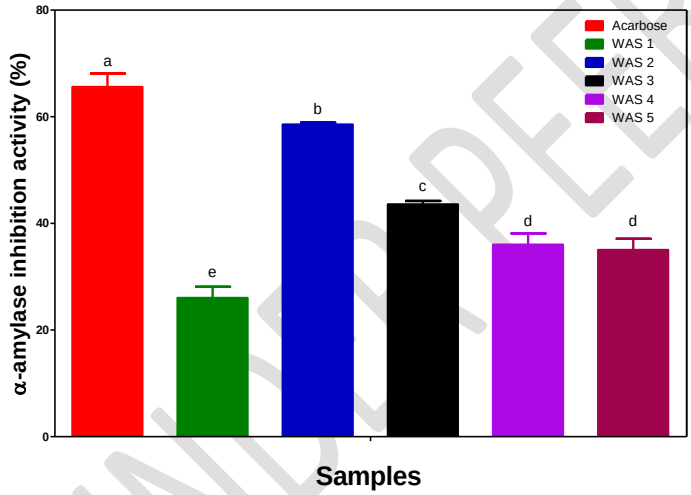

Fig .5: *In vitro* α-amylase inhibition activities of cookies
Bars (n=3) with different letter are significantly different ($p<0.05$).
Key:WAS 1 = 100% wheat (commercial flour); WAS 2 = 45% wheat flour + 20% sweet potato flour + 35% African yam bean flour; WAS 3 = 40% wheat flour + 30% sweet potato flour + 30% African yam bean flour; WAS 4 = 35% wheat flour + 40% sweet potato flour + 25% African yam bean flour; WAS 5 = 30% wheat flour + 50% sweet potato flour + 20% African yam bean flour

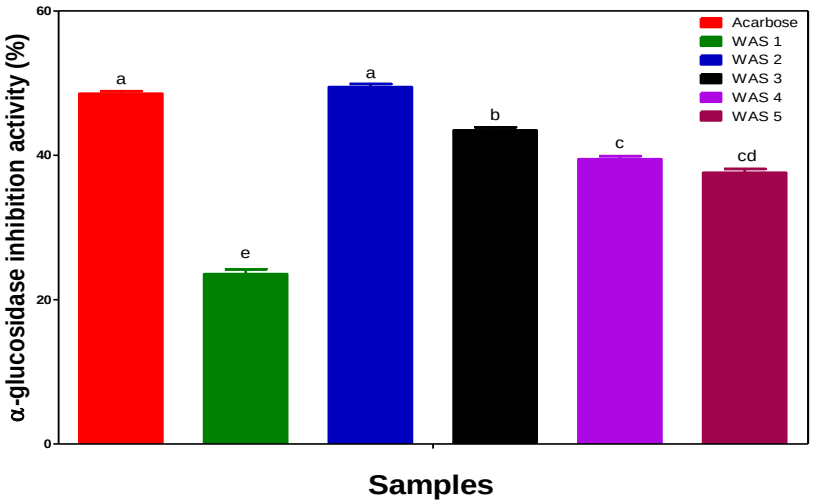

Fig 6: *In vitro* α-glucosidase inhibition activities of cookies
Bars (n=3) with different letter are significantly different ($p<0.05$).
Key:WAS 1 = 100% wheat (commercial flour); WAS 2 = 45% wheat flour + 20% sweet potato flour + 35% African yam bean flour; WAS 3 = 40% wheat flour + 30% sweet potato flour + 30% African yam bean flour; WAS 4 = 35% wheat flour + 40% sweet potato flour + 25% African yam bean flour; WAS 5 = 30% wheat flour + 50% sweet potato flour + 20% African yam bean flour

However, the result (Fig 6) showed no significant difference ($p>0.05$) between cookie sample WAS 2 and acarbose, a well-known anti-diabetic drug. Hence, the cookie sample WAS 2 has potential to be used to modulate the type 2 diabetes. The cookie sample WAS 2 obtained from 45% wheat flour + 20% sweet potato flour + 35% African yam bean flour has improved inhibition of α-glucosidase and this might be due to its higher protein content as well as its better glutamic amino acids, when compared to other samples [28].

3.5 Physical properties of cookies

The result of the physical properties of cookies produced from wheat, sweet potato and African yam bean flour blends was shown in Table 4. The weights of the cookie samples WAS 2 and 5 were significantly ($p<0.05$) highest (14 g) when compared to WAS 1 (12.72 g). However, there seemed no significant difference between the diameters and thickness of the cookie samples, which ranged between 5.23 – 5.52 mm and 0.43 – 0.50 mm, respectively. The increase in thickness were noticed with increasing levels of substitution with sweet potato but contrary trend was recorded on spread ratio. Spread ratio has been reported to be affected by the competition of ingredients for the available water [4, 7]. Other functional properties such as, proteins and fat might have also affected the spread ratio. It can therefore be deduced that both protein and fat content in the African yam bean flour had effect on the spread ratio of the cookies. The observable occurrences on the spread ratio of cookies in this current study seemed not to be due to competition over available water by ingredients as both African yam bean and sweet potato flours absorbed water during dough mixing. The observed noticeable differences might be traced to the protein and fat contents of the blends and thus affected the rise in the

cookies during the baking process. Above all, the composite cookies showcased attractive physical properties.

Table 4: Physical properties of cookies

Samples	Weight (g)	Diameter (cm)	Thickness (cm)	Spread ratio
WAS 1	12.72±0.59^{c}	5.23±0.07^{ab}	0.50±0.10^{a}	10.46±2.51^{ab}
WAS 2	14.67±1.08^{a}	5.34±0.20^{a}	0.43±0.03^{ab}	12.42±1.21^{c}
WAS 3	13.93±0.01^{b}	5.39±0.14^{a}	0.46±0.09^{ab}	11.72±2.06^{b}
WAS 4	13.99±0.58^{b}	5.44±0.09^{a}	0.45±0.03^{ab}	12.09±0.97^{b}
WAS 5	14.09±0.79^{a}	5.52±0.15^{a}	0.59±0.10^{a}	9.36±2.62^{a}

Means (n=3) with different letter in the column are significantly different ($p<0.05$).
Key:WAS 1 = 100% wheat (commercial flour); WAS 2 = 45% wheat flour + 20% sweet potato flour + 35% African yam bean flour; WAS 3 = 40% wheat flour + 30% sweet potato flour + 30% African yam bean flour; WAS 4 = 35% wheat flour + 40% sweet potato flour + 25% African yam bean flour; WAS 5 = 30% wheat flour + 50% sweet potato flour + 20% African yam bean flour

3.6 Sensory attributes of cookies

The mean scores for sensory qualities of cookies given in Table 5 revealed that there were significant differences ($p\leq0.05$) in the appearance, aroma, taste, texture, mouth feel and overall acceptability of the cookies.This current result agreed with the past report that baked goods using sweet potato as ingredient, provided one of the most attractive possibilities because it increased dough yield and contributed to attractive crumb and crust of the baked products [14]. It has also been reported that African yam bean flour also added to the improved crumb quality of the baked products [29]. All these have been indicated by the reaction of panelist in the evaluation of the appearance of the cookies (Table 5). Meanwhile, noticeable changes in appearance from light brown to darker shades of brown could be associated to non-enzymatic browning reactions (Maillard reactions) between reducing sugar molecules and lysine. Legumes, such as African yam beans have been reported to be rich in lysine, which produced darker shades of brown colours[9].

Mean for taste revealed that the WAS had highest score (7.40), which is not significantly different from the WAS 3 with 7.33, while WAS 4 (5.17) had least score. The remarkable feeling grits (texture and mouthfeel) in cookies WAS 2 could be associated to its small particle size. These grits could probably be from African yam bean fraction of the composite flour, which possessed high tendency to absorb water to make cookies with higher proportion of both dampness and crispness. The mean overall acceptability of the cookies revealed that the WAS 2 from 45% wheat flour + 20% sweet potato flour + 35% African yam bean flour was overall acceptable (7.80) while the least acceptable product is sample WAS 1 from 100% wheat flour (6.03). The high acceptability of WAS 2 might be probably due to its improved appearance, taste and mouthfeel.

Table 5: Sensory attributes of different cookies

Samples	Appearance	Aroma	Taste	Texture	Mouthfeel	Overall

						acceptability
WAS 1	6.47[b]	6.03[ab]	5.70[c]	5.53[b]	5.30[b]	6.03[b]
WAS 2	7.37[a]	6.63[a]	7.40[a]	7.40[a]	7.90[a]	7.80[a]
WAS 3	6.63[b]	6.03[ab]	7.33[a]	7.77[a]	7.20[a]	6.83[b]
WAS 4	6.40[b]	6.07[ab]	5.17[c]	5.27[b]	5.20[b]	5.80[c]
WAS 5	6.40[b]	6.07[ab]	6.83[b]	6.53[b]	5.03[bc]	6.27[b]

Means (n=3) with different letter in the column are significantly different ($p<0.05$).
Key:WAS 1 = 100% wheat (commercial flour); WAS 2 = 45% wheat flour + 20% sweet potato flour + 35% African yam bean flour; WAS 3 = 40% wheat flour + 30% sweet potato flour + 30% African yam bean flour; WAS 4 = 35% wheat flour + 40% sweet potato flour + 25% African yam bean flour; WAS 5 = 30% wheat flour + 50% sweet potato flour + 20% African yam bean flour

4.1 CONCLUSION

The findings from this study have shown thewheat, sweet potato and African yam bean composite flours as potential ingredients for the production of acceptable quality and nutritious cookies. The cookies produced from blends of 45% wheatflour + 20% sweet potato flour + 35% African yam bean flours gave the best products due to its high crude protein, high crude fibre, low crude fat contents as well as their overall acceptability to the consumers, when compared to others. However, all the composite cookies possessed rich amino acid profiles, improved antioxidant properties and enhanced inhibition potentials against the activities of the dual carbohydrate hydrolyzing enzymes that have been implicated in the pathogenesis of diabetes. This therefore, showed that they could be helpful in reducing insulin demand, improving satiety, improving blood glucose control in diabetic people.In any case, adoption of this cookie production technology would result in production of better protein and fibre-enriched products to the ever-increasing number of diet-conscious consumers. Moreover, consumption of functional cookies from composite flour blends could be nutritionally more superior to those from whole-wheat flour in terms of improving the nutritional status of the consumers; thus, serving as a vehicle for protein and other nutrient fortification in Nigeria.

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