Antioxidant Properties of a Novel Food Product,*Aadun* (Pudding) from Roasted Maize-African Yam Bean-Peanut Flours Enhanced Its Anti-Hyperglycemia Potentials in Wistar Rats

ABSTRACT

The study aimed to investigate the antioxidant properties of a novel food product, *aadun* (pudding) from roasted maize-african yam bean-peanut flours as it enhanced the anti-hyperglycemia potentials of the cookies in Wistar rats.*Aadun* was produced from a blend of roasted maize, African yam bean (AYB), andpeanut flour at different ratios (100%; 70:20:10%; 60:30:10%; and 50:40:10%) and labelled WKB 1, WKB 2, WKB3, and WKB 4, respectively, while a commercial sample (100%) was used as a positive control. The *aadun* was investigated for its proximate composition, titrable acidity, amino acid profile, in vitro antioxidant, in vivo glycemic index, and consumer acceptability. The proximate composition result showed that the protein and fiber content ranges were 11.90–13.91% and 2.68–3.30%, respectively. Its bioactivities were improved, as indicated by its hydrophobic amino acids and biological value (~71.96–87.57%), the respective hydroxyl radical scavenging and iron chelation activities of the *aadun* were 18.30–69.90 and 16.40–77.9% with sample WKB 3 (60:30:10%; maize:AYB:peanut) and the control sample having the highest and least activities, respectively. The sample WKB 3 had the highest DPPH and highest ferric-reducing properties. The aadun had a low glycemic index (GI) of 34.14–45.45%. Most interesting is that the panellists generally accepted all the aadun samples compared tothe commercial *aadun*.

Keywords: *Aadun;* African yam bean; amino acid; antioxidant; hyperglycemia

1. INTRODUCTION

Hyperglycemia, also known as high blood glucose, occurs when too much sugar is inthe blood. For instance, this happens when the body has very little insulin or if the body is unable to use insulin properly; hence, the condition is most often linked with diabetes [1]. This condition occurs when the sugar level in the blood is >125 and >180 mg/dL during fasting and 2 h postprandial, respectively. Thus, a diabetic is someone who has a fasting blood glucose of >125 mg/dL [1]. However, when hyperglycemia is left untreated, it could lead to many serious life-threatening complications that include but are not limited to damage to the eye, kidneys, nerves, heart, and peripheral vascular system [2]. Findings show that chronic hyperglycemia occurs in uncontrolled diabetes, which leads to biochemical changes that consequently cause changes in the hemodynamics of the retina vessels and cause chronic hypoxia, a deficiency in the amount of oxygen reaching the tissues [2,47,47-49].

Inflammation is a complex set of interactions among soluble factors and cells that could arise in any tissue in response to traumatic, infectious, toxic, or autoimmune injury [3]. However, if targeted destruction and assisted repair are not properly phrased, inflammation could lead to persistent tissue damage by leukocytes, lymphocytes, and collagen [3]. However, a study also reported the association between chronic inflammatory diseases and infections with an increased risk of cardiovascular disease [4]. Sometimes, inflammation serves as a natural defense mechanism against several damaging factors, including chronic diseases such as hyperglycemia due to the high production of reactive oxygen species [4]. This could have been the reason for the inflammation observed in several other chronic diseases including Alzheimer's disease and cancer [4].

Aadun, a common snack obtained from roasted maizeflour, pepper, and salt after being thoroughly mixed with palm oil (to obtain a uniform product) is a popular food product among Yoruba tribes in Nigeria [5].*Aadun* is a good source of carbohydrates, phosphorus, and magnesium, but it is low in protein and often sold under minimal packaging conditions, which might lead to its rapid deterioration [6]. Interestingly, the use of locally available food sources such as maize and defatted African oil bean seed for the production of *aadun* has been widely reported [7]. Hence, this research intended to explore the use of maize, African yam beans (AYB), and peanuts after roasting in the production of *aadun*. Recently, up to 40% of AYB was used together with maize without negatively affecting other sensory properties of the produced *aadun* [8].

Maize *(Zea mays)* is a major staple crop worldwide, accounting for 37.2% of total worldwide cereal production [9]. It is regarded as one of the most important food crops in the world, with global cultivation on acreage of 228 million ha in 2016 [10]. It contains large amounts of bioactive compounds, such as carotenoids, ferulic acid, and anthocyanins, with many therapeutic properties as well as fat-soluble vitamins [11].

African yam bean *(Sphenostylis stenocarpa)*belongs to the family, *Papilionacea*, which is sometimes classified in the sub-family *Leguminosae* [12]. It is an edible, underutilized grain legume widely cultivated in Africa that is used for human and animal nutrition [13]. African yam bean is rich in protein (19.5%), carbohydrates (62.6%), fat (2.5%), vitamins, and minerals [13]. The protein is made up of over 32% essential amino acids, while its amino acid spectrum indicates that lysine (which is lacking in maize) and methionine are the most predominant ones [12].Peanut *(Arachis hypogae)*, also known as groundnut, is a legume crop grown mainly for its edible seeds. It is generally included among the oilseeds due to its high oil (~57%) content [14]. Apart from its high oil content, it contains many other functional compounds, such as protein (26%), polyphenols, antioxidants, vitamins, and minerals, which could be added as a functional ingredient to many processed foods [15].

Composite flour is a mixture of flour that is intended to totally or partially replace wheat flour in bakery and pastry products [16]. It is a mixture of flour from some other crops with or without wheat flour [16]. Composite flour is considered advantageous in developing countries as it reduces the importation of wheat flour and encourages the use of locally grown crops [17,18]. For instance, different varieties of crops such as breadfruit and breadnut [19], rice [20], Bambara groundnut [21], soybean [22], tiger nut tuber [23], sweet potato [24], and African walnut [25], have been used in the preparation of several composite flour formulations. This study investigates the antioxidant and anti-hyperglycemia properties of *aadun* produced from roasted maize, African yam bean, and peanut composite flour

2. MATERIALS AND METHODS

2.1. Materials

The maize, African yam bean, and peanut grains were obtained from King's Market, Ogbomoso, Nigeria, and authenticated at the Department of Crop, Soil and Pest Management of the Federal University of Technology, Akure, Ondo State, Nigeria. The chemicals and reagents used for this study were purchased from Sigma-Aldrich, London, United Kingdom, and were all certified and analytically graded before use.

2.2. Production of maize, African yam bean, and peanut flour

The maize, African yam bean, and peanut flour were obtained according to the previously described methods [26–28]. Briefly, the raw seeds were sorted and cleaned, roasted in an oven for 20 min at 180 °C, dehulled and separately milled using a Binatone kitchen blender (Binatone, mode BLG 4O2, Zhongshan, Haishang, China), and the resultant flour sieved to obtain a uniform size of 40 µm. The individual flours were then formulated into composite flour blends as shown in Table 1**2.3. Formulation of Composite Flour Blends**

The composite flour blends were formulated as stipulated:

Table 1. Composite flour formulations.

Samples	Formulations	Maize	African Yam Bean	Peanut	Total (%)
WKB 1	Maize	100	-	-	100
WKB 2	Maize + AYB + Peanut	70	20	10	100
WKB 3	Maize + AYB + Peanut	60	30	10	100
WKB 4	Maize + AYB + Peanut	50	40	10	100

Commercial product	100	-	-	100

2.4. Production of Aadun

Aadun was prepared according to the previously described method [7]. Briefly, the composite flour blend was manually and properly mixed in a bowl with salt, ground pepper, and palm oil. It was then moulded and packaged for further analysis.

2.5. Proximate Composition Analysis

Proximate compositions of *aadun* were determined as described by AOAC 14.1.2.4.6.9 [29]. The carbohydrate content was determined by the difference (100-the sum of the content of protein, fat, ash, and moisture), while the energy value was calculated using the Atwater factor (fat × 9 + carbohydrate × 4 + protein × 4 kcal/100 g).

2.6. Amino Acid Analysis

Amino acid profiles of aadun were determined according to the modified method [30]. Briefly, the food sample was placed in a hydrolysis ampoule and then dried under vacuum using a Savant SpeedVac (Fisher Scientific, Lougbborough, UK). Approximately 100 µL of 6 N HCL was placed in the lower part of the ampoule, frozen in a dry ice/ethanol bath, attached to a vacuum system via ¼″ ID x $^{5}/_{8}$″ OD Tygon tubing, then slowly thawed and evacuated to <150 mTorr. An oxygen/methane flame was used to seal the neck of the tube at the constriction. After hydrolysis and acid removal, samples that contained 0.5–10 µg of protein were reconstituted with 60–200 µL of Na-S sample buffer and the amino acid composition was then finally analyzed.

2.7. Antioxidant Assays

The aadun was analyzed for their hydroxyl (OH) and DPPH radical scavenging, chelation of metal ions, and ferric-reducing antioxidant power (FRAP) activities according to their respective methods as modified and previously described [30].

2.8. *In Vivo* Glycemic Index and Blood Glucose Concentration

The glycemic index of *aadun* and the blood glucose concentrations in rats fed with the *aadun* samples were investigated using the previously described method [31]. About thirty (30) Wistar Albino rats, having body weights of 140 to 150 g, were purchased and grouped into 5 groups with 5 rats in each group. They were caged individually in a location where the environmental conditions were controlled and were provided food and water ad libitum. The rats were subjected to acclimatization of the new living location for 7 days. After this period, the animals were weighed and underwent overnight fasting (12 h). Their blood glucose was taken at '0' time from the tail vein before giving them 2.0 g of *aadun* samples and glucose (control) for consumption (for like 25 min). Afterwards, their serum glucose levels were measured according to the manufacturer's regulations (Automatic glucose analyzer, Accu-chek Active Diabetes monitoring kit; Roche Diagnostic, Indianapolis, IN, USA) at 0 to 120 min with 15-min intervals. The glycemic response was calculated as the Incremental Area under the Blood Glucose Curve (IAUC), which is geometrically obtained from the graph of concentration vs. time.

2.9. Microbial Analysis

Microbial loads of the *aadun* samples were investigated as previously described [32]. Briefly, 10 g of the food sample was mixed aseptically using a sterile spatula into a sterile blender jar. Then, 90 mL of 0.1% buffered peptone water (BPW) was added to the sample and then blended at a speed of 15,000–20,000 rpm for 90 s. The homogenate was mixed by gentle shaking and 1.0 mL was pipetted and dispensed into a tube containing 9 mL of the 0.1% buffered peptone water. It was then mixed carefully by aspirating 10 times with a fresh pipette and 1.0 mL from the first dilution was transferred with the same pipette to the second dilution tube containing 9 mL 0.1% BPW. It was mixed by shaking 25 times in a 30 cm arc to obtain (10^{-2}) dilution. This procedure was repeated with 8 tubes to make 8 serial dilutions. After this, 1 mL of the homogenate was pipetted from each dilution into each of the appropriately marked duplicate Petri dishes. Then, 15 mL of the nutrient and potato dextrose agar was poured into each plate and kept at 45 ± 1 °C in a water bath. The sample dilution and agar media were thoroughly and uniformly mixed by alternate rotation and back-and-forth motion of plates on flat-level surfaces while the mixture was left to solidify. The solidified plates were then inverted and promptly incubated for 48 ± 2 h at 35 °C.

2.10. Physical Properties Determination

The aadun was manually but carefully molded into a rectangular shape and then investigated for their weight, diameter, thickness (width), and spread factor (diameter/thickness) using the previously described methods [33].

2.11. Evaluation of Sensory Attributes

The aadun was given some codes and presented to 50 semi-trained panellists for evaluation of their texture, appearance, aroma, taste, mouth feel, etc. using a Hedonic scale of 1 to 9, where 1 = dislike extremely and 9 = like extremely as described [33].

2.12. Statistical Analysis

All determinations were carried out in triplicates. Data were subjected to analysis of variance (ANOVA) using SPSS (version 21, Chicago, United States of America), while means were separated using the Duncan Multiple Range Test (DMRT) at a 5% level of significance ($p < 0.05$).

3. RESULTS AND DISCUSSION

3.1. Proximate Composition of Aadun Samples

The proximate compositions of *aadun* produced from roasted maize, African yam bean,and peanut composite flour blendsare shown in Table 2. The range of moisture contents is given as 4.28–4.89%, which is lower than the 5.53–10.52% reported for *aadun* from the maize-groundnut mixture [34] but similar to the 4.79–9.63% reported for *aadun* from the maize-defatted African oil bean seed mixture [7].

Table 2. Proximate composition of composite *Aadun*(maize pudding) (%).

Sample/ Parameters	Moisture	Fat	Fiber	Ash	Protein	Carbohydrate	Titrable Acidity (%)
WKB 1	4.28 ± 0.36 [c]	22.90 ±0.06 [ab]	2.68 ± 0.30 [b]	1.00 ± 0.04 [c]	11.90 ± 0.12 [c]	57.26 ± 0.11 [ab]	1.32 ± 0.01 [b]
WKB 2	4.74 ± 0.06 [b]	20.64 ± 0.07 [c]	3.20 ± 0.06 [a]	3.59 ± 0.14 [a]	12.51 ± 0.05 [b]	55.33 ± 0.09 [c]	1.34 ± 0.01 [b]
WKB 3	4.89 ± 0.05 [a]	20.59 ± 0.01 [c]	3.30 ± 0.00 [a]	2.12 ± 0.05 [b]	13.21 ± 0.05 [a]	55.89 ± 0.08 [c]	1.29 ± 0.08 [b]
WKB 4	4.19 ± 0.11 [c d]	20.74 ± 0.03 [c]	3.00 ± 0.05 [ab]	2.91 ± 0.01 [b]	13.91 ± 0.01 [a]	55.09 ± 0.14 [c]	1.11 ± 0.01 [c]
Commercial sample	4.33 ± 0.11 [c]	24.72 ± 0.03 [a]	2.96 ± 0.05 [b]	3.61 ± 0.01 [a]	13.91 ± 0.01 [a]	58.69 ± 0.14 [a]	1.46 ± 0.03 [a]

Means (n = 3) with different letters in the column are significantly different ($p < 0.05$). **Key:**WKB 1 = 100%Maize; WKB 2 = 70%Maize + 20%AYB + 10%Peanut; WKB 3 = 60%Maize + 30%AYB + 10%Peanut; WKB 4 = 50%Maize + 40%AYB + 10%Peanut.

The ash content of the *aadun* was between 1.00 and 3.59% with the highest and least values significantly ($p < 0.05$) obtained for samples WKB 2 (70:20:10%; maize:AYB:peanut) and WKB 1 (100% maize). It is interesting to note that the current results fall within the ranges of the 2.73–4.62% reported [34] for aadun made from maize and groundnut. The fat content ranged from 20.59 to 22.90%, with sample WKB 3 (60:30:10%; maize:AYB:peanut) and WKB 1 having the least and highest contents, respectively. Although the present values obtained in the current study were similar to the 10.34–23.50% reported for aadun from maize and groundnut [34], another study [35] reported a fat content of 34.7–40.6% for aadun from a maize-groundnut-crayfish flour blend. The higher content in the latter study could be a result of the composition of the animal source (crayfish) when compared to the current study.

The fiber content of the aadun ranged from 2.68 to 3.30%, with sample WKB 3 having the highest fiber content. A previous study has revealed that AYB is a good source of fiber, such as hemicellulose, cellulose, and lignin [36]. The *aadun* samples had significant ($p < 0.05$) protein content of 11.90–13.91%, whereas samples WKB 1 and WKB 4 (50:40:10%; maize:AYB:peanut) had the least and highest contents, respectively. The result compared to the 10.09–14.98% obtained for aadun from maize and groundnut [34] but lower than 10.0–22.7% reported for aadun from the maize-groundnut-crayfish blend [35], respectively. The composite aadun had significantly ($p < 0.05$) higher protein than the control aadun (100% maize), which could be due to the important presence of AYB, which has high nutritional quality.

3.2. Amino Acid Profiles of Aadun Samples

The amino acid profile of aadun produced from roasted maize, African yam bean,and a peanut flour blendwith a control sample is presented in Table 3a. The snacks have higher leucine (6.19–7.87 g/100 g), lysine (1.26–3.39 g/100 g), methionine (2.26–2.99 g/100 g), histidine (2.67–2.98 g/100 g), and glycine (2.57–3.61 g/100 g) than the control sample (6.15, 1.05, 2.01, 2.10 and 2.24 g/100 g, respectively). Notably, glutamic acid, the most concentrated amino acid (16.46–19.67 g/100g) in aadun, is very useful for maintaining the acid/alkaline balance in the body after conversion to glucose or sugar [24]. Sample WKB 1 and the commercial sample both contained the same amount of tryptophan (0.98 g/100 g) and this might be due to the flours being produced from 100% maize formulations only.

Table 3. (a) Amino acid profiles of *Aadun* samples (g/100 g). (b) Estimated and predicted nutritional quality of aadun samples.

(a)

Samples/ Amino Acids	**WKB 1**	**WKB 2**	**WKB 3**	**WKB 4**	**Commercial Sample**	**Average ± Std**		**#LSD ($p < 0.05$)**
Leucine	6.19	7.59	6.83	7.87	6.15	6.93	0.86	0.37
Lysine	1.26	2.98	3.39	3.17	1.05	2.37	0.18	1.00
Isoleucine	3.03	3.89	3.75	3.11	3.01	3.36	0.39	0.33
Phenylalanine	1.71	2.57	3.04	3.48	1.11	2.38	0.23	1.00
Valine	3.38	3.87	4.24	3.91	3.25	3.73	0.14	1.00
Methionine	2.26	2.71	2.93	2.99	2.01	2.58	0.10	0.15
Tryptophan	0.98	1.34	1.31	1.11	0.98	1.14	0.12	1.00
Threonine	3.21	3.30	3.79	3.99	3.23	3.50	0.34	1.00
Tyrosine	2.11	2.74	3.12	3.35	2.09	2.68	0.44	1.00
Cystine	1.70	2.38	2.98	2.96	1.74	2.35	0.32	1.00
Histidine	2.67	2.98	2.92	2.97	2.10	2.73	0.09	0.09
Alanine	3.62	3.21	3.22	3.47	3.22	3.35	0.55	1.00
Proline	5.69	5.97	7.49	8.38	5.41	6.59	0.41	1.00
Glutamic acid	16.46	18.03	19.24	19.67	16.28	17.94	1.78	1.00
Serine	4.94	3.41	3.98	3.69	4.32	4.09	0.54	1.00
Aspartic acid	4.77	5.30	6.81	6.45	4.19	5.50	0.22	1.00

Glycine	2.57	3.61	3.22	3.28	2.24	2.98	0.51	1.00
Arginine	4.43	3.87	3.95	3.85	4.17	4.05	0.54	1.00
TEAA	29.12	35.10	36.15	36.45	27.06	32.78	1.86	1.00
TNEAA	41.86	44.65	50.06	51.32	40.49	50.06	1.98	1.00
TAA	70.98	79.75	86.21	87.77	66.55	78.24	3.39	1.00

(b)

***Aadun* Samples Parameters**	**WKB 1**	**WKB 2**	**WKB 3**	**WKB 4**	**Commercial Sample**
HAA	30.67	36.27	38.91	40.62	28.97
AAA	4.80	6.65	7.47	7.94	4.18
Arg/Lysine	3.52	1.30	1.17	1.22	3.97
BCAA	12.60	15.35	14.82	14.89	12.41
NCAA	21.23	23.33	26.05	26.12	20.47
PER(g/100g)	3.32	3.77	4.84	4.91	3.11
EAAI	0.63	0.85	0.89	0.89	0.61
EAAI (%)	63.00	85.12	89.42	89.83	61.39
BV (%)	72.47	83.22	86.28	87.57	71.96
Fischer Ratio	2.63	2.31	1.98	1.87	2.97

TEAA—Total essential amino acids, **TNEAA**—Total non-essential amino acid, **TAA**—Total amino acid., #**LSD** = Least significant difference. **HAA**—Hydrophobic amino acid, **AAA**—Aromatic amino acid **BCAA**—Branched-chain amino acid, **NCAA**—Negatively charged amino acid **PER**—Protein efficiency ratio, **EAAI**—Essential amino acid index, **BV**—Biological value.

Key: WKB 1 = 100% Maize; **WKB 2** = 70% maize + 20% AYB + 10% peanut; **WKB 3** = 60% maize + 30% AYB + 10% peanut; **WKB 4** = 50% maize + 40% AYB + 10% peanut.

The estimated and predicted nutritional quality of the formulated aadun and control sample is presented in Table 3b. The high *Arg* level is significant because of its cardiovascular health benefits by improving the hypocholesterolemic status of any arginine-containing food product. Therefore, the present result showed that the aadun with the *Arg/Lys* ratio of 1.17–3.97 g/100 g may enhance the cardiovascular health of individuals. It was also observed that sample WKB 1 and the commercial sample had the highest value (3.52; 3.97 g/100 g, respectively) when compared to the formulated samples. The improved antioxidant and anti-hyperglycemia of the aadun might be due to its improved HAA, AAA, and BCAAs (Table 3b).

The predicted protein efficiency ratio (PER) of the samples ranged from 3.32 to 4.91 g/100g, with higher values in WKB 4 (50:40:10%; maize:AYB:peanut) and WKB 3 (60:30:10%; maize:AYB:peanut) when compared with the control sample (3.11 g/100g). In addition, the essential amino acid index (EAAI) and biological values (BV) of the aadun ranged from 63.00–89.83 and 72.47–87.57% when compared to the control sample (61.39 and 71.96%), respectively. A food with nutritional qualities of >2.7 PER, >70% BV, and >0.70 EAAI has been reported to possess good nutritional status [24]. Hence, the nutritional status obtained in the current study showed that the formulated aadun contained an appreciable amount of protein, which is adequate to give the daily amounts required by the consumers.

3.3. In Vitro Antioxidant Properties of Aadun Samples

The present OH radical scavenging values significantly ($p < 0.05$) ranged from 18.30 to 69.9% (Figure 1), with sample WKB 3 (60:30:10%; maize:AYB:peanut) and WKB 1 (100% maize) having the highest and least values, respectively. The higher value exhibited by sample

WKB 3 could be an indication of the contribution from its higher protein (Table 2) and hydrophobic amino acids (Table 3b); hence, WKB 3 could serve as a potential antioxidant agent by inhibiting the interaction of hydroxyl radicals with DNA. The scavenging potential of the *aadun* sample to quench hydroxyl radicals is directly related to its prevention of lipid peroxidation [37].

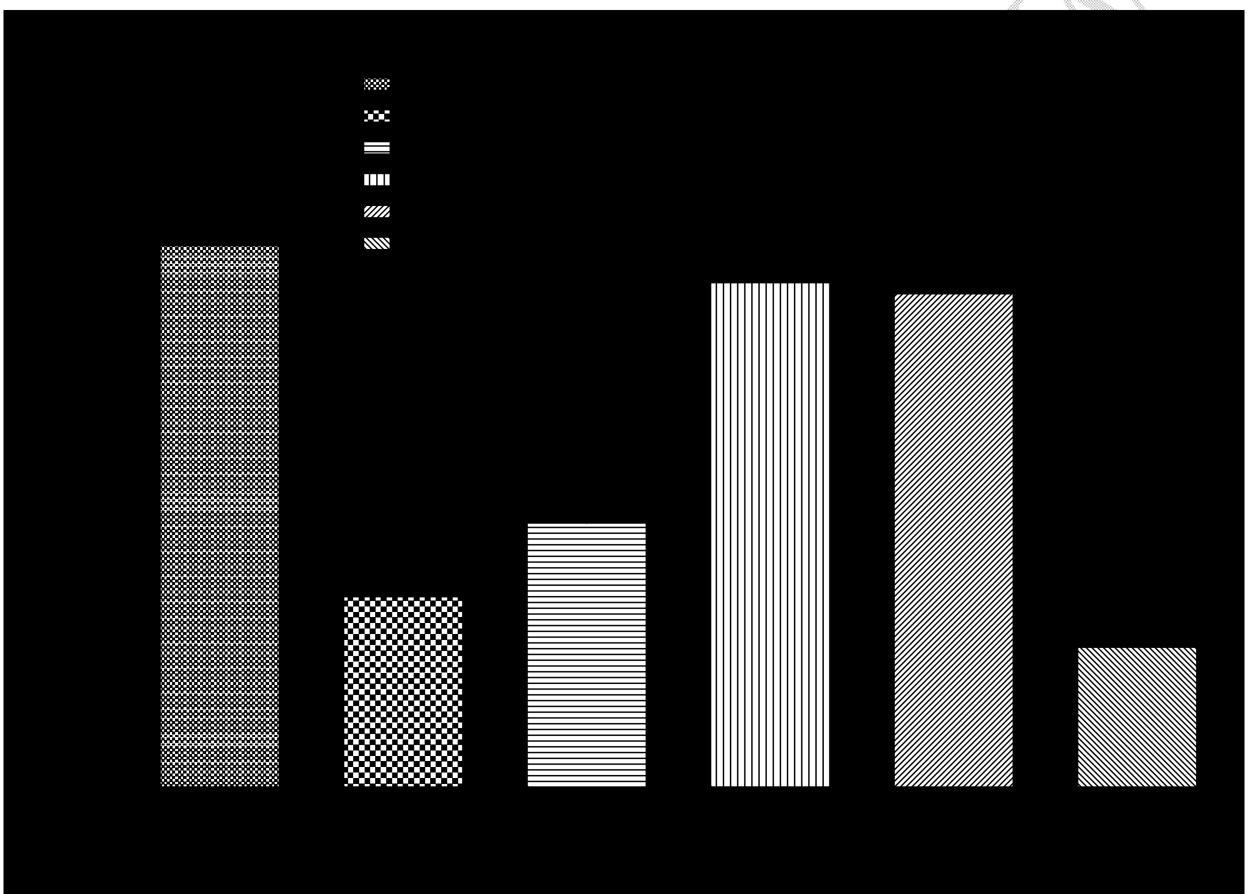

Figure 1. Hydroxyl (OH) scavenging potential of different *aadun* samples.

Bars (n = 3) with different letters are significantly different ($p < 0.05$). Key: WKB 1 = 100% maize; WKB 2 = 70% maize + 20% AYB + 10% peanut; WKB 3 = 60% maize + 30% AYB + 10% peanut; WKB 4 = 50% maize + 40% AYB + 10% peanut.

The DPPH values of the *aadun* samples presented in Figure 2, with WKB 3 having the significant ($p < 0.05$) highest value. This revealed that the levels of substitution of maize with

AYB during *aadun* production has a great influence on the DPPH radical scavenging abilities of the produced aadun products. This is because the awareness of AYB as an important and non-expensive means of getting antioxidants has been known and exploited during the management of several chronic diseases, like cancer, CVD, etc. [38]. Another interesting observation of the present result is that an increase in the DPPH capacities is observed as the level of substitution with AYB increases.

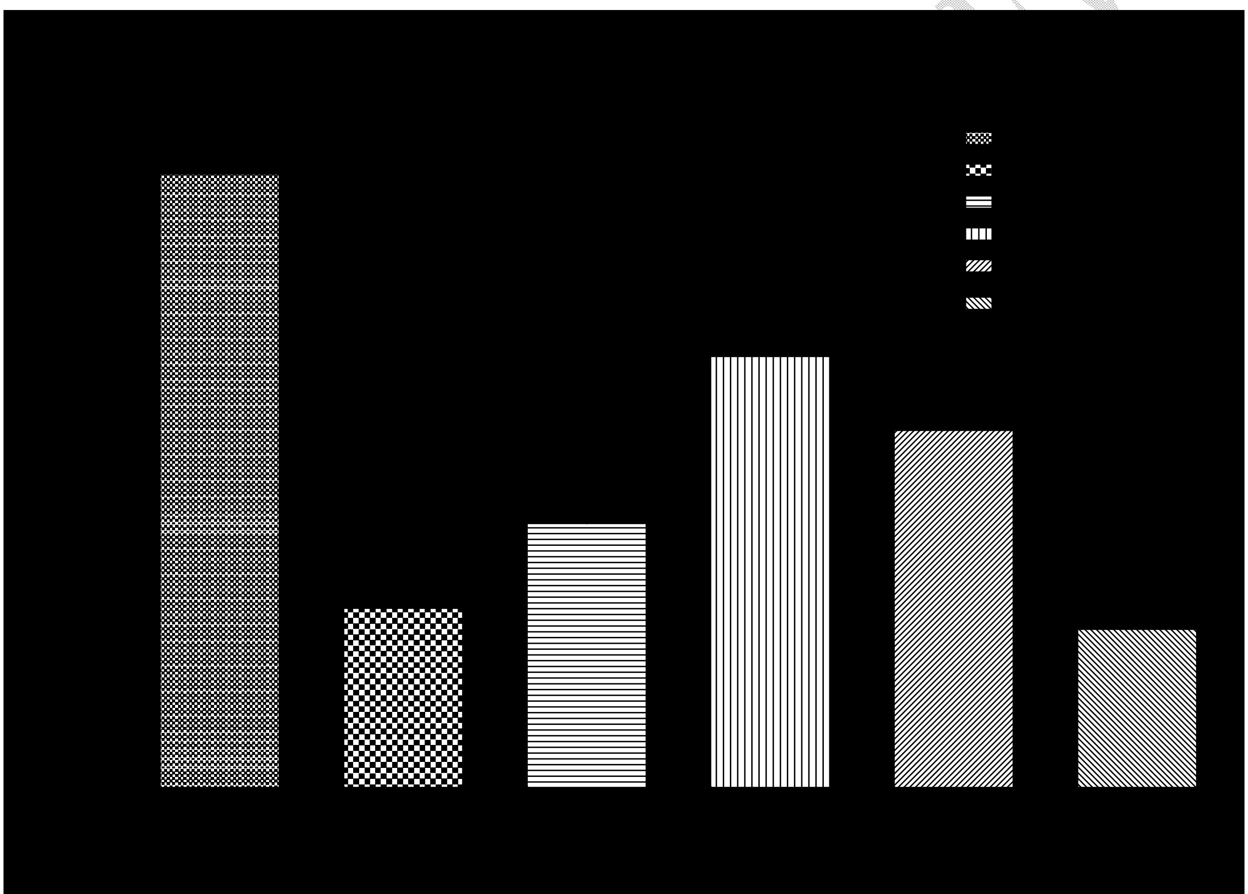

Figure 2. DPPH scavenging potential of different *aadun* samples.

Bars (n = 3) with different letters are significantly different ($p < 0.05$). Key: WKB 1 = 100% maize; WKB 2 = 70% maize + 20% AYB + 10% peanut; WKB 3 = 60% maize + 30% AYB + 10% peanut; WKB 4 = 50% maize + 40% AYB + 10% peanut.

The iron chelating value of the aadun samples ranged from 16.4 to 77.9%, as shown in Figure 3. As previously observed, the highest value reported for WKB 3 might be due to its high level of AYB and/or its higher hydrophobic and aromatic amino acids (Table 3b). In addition, the study has shown that the chelating agents act as secondary antioxidants when they stabilize transition metals in living systems, and this phenomenon has been found to be important in retarding radical degradation by inhibiting radical generation [39].

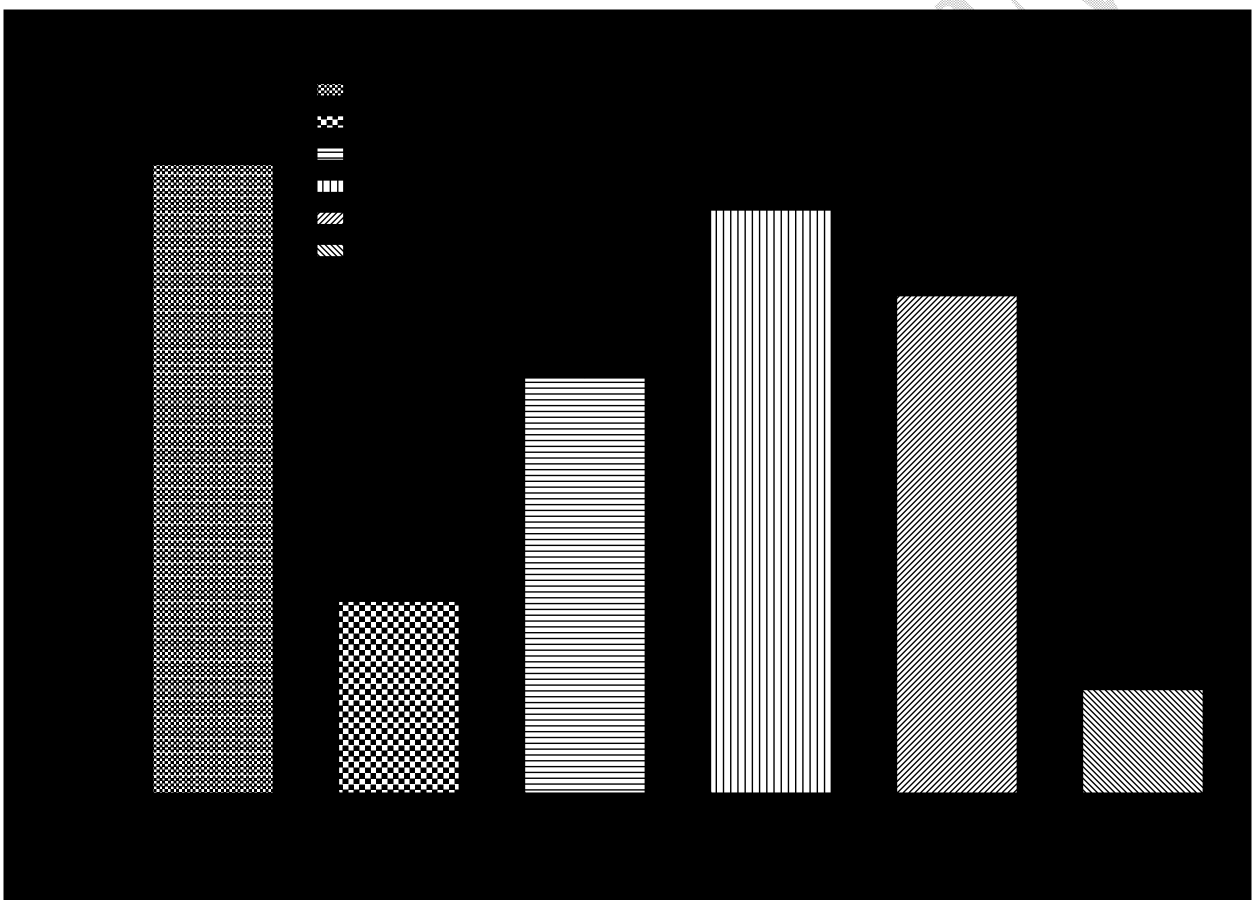

Figure 3. Metal (Fe^{2+}) chelation properties of different *aadun* samples.

Bars (n = 3) with different letters are significantly different ($p < 0.05$). Key: WKB 1 = 100% maize; WKB 2 = 70% maize + 20% AYB + 10% peanut; WKB 3 = 60% maize + 30% AYB + 10% peanut; WKB 4 = 50% maize + 40% AYB + 10% peanut.

The FRAP activities of the *aadun* samples are presented in Figure 4. The result revealed that as the level of substitution of maize with AYB increased, there was an increase in FRAP values. For instance, sample WKB 3 had the highest content, at 0.56 mg/g, when compared to sample WKB 1 (100% maize aadun), which had 0.24 mmol Fe^{2+}/mg. The incorporation of AYB and peanut could have contributed to the improvement of the reducing power of the *aadun* samples, through the efficient mechanism of hydrogen atom transfer for the ferric ion [40]. In addition, all the composite *aadun* samples still had significantly higher ($p < 0.05$) reducing powers than the control sample (Figure 4). The higher values obtained reflect that the sample WKB 3, among the formulated samples, had the potential of reductant formation during the free radical reactions and the potential to stabilize or terminate the reaction chains [41].

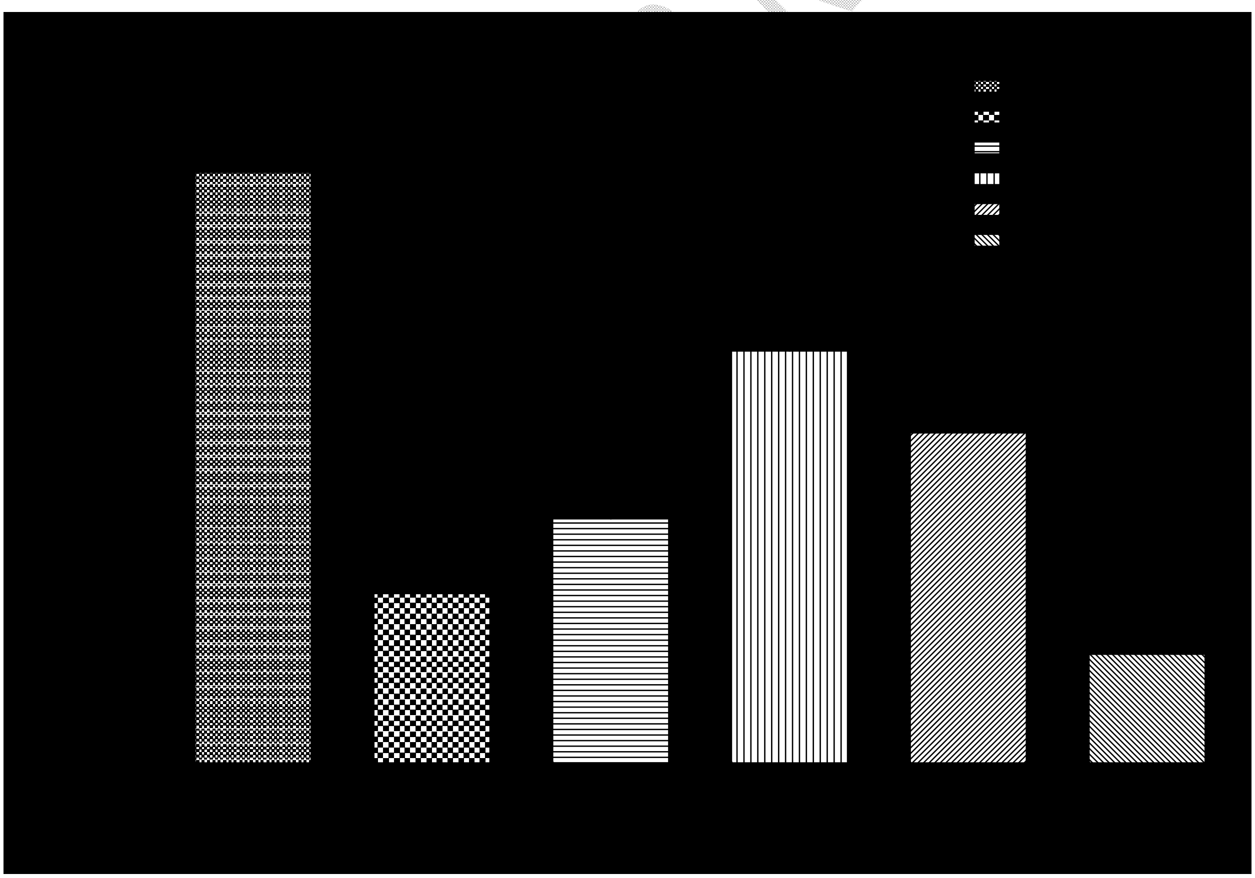

Figure 4. Ferric ion reducing properties of different *aadun* samples.

Bars (n = 3) with different letters are significantly different ($p < 0.05$). Key: WKB 1 = 100% maize; WKB 2 = 70% maize + 20% AYB + 10% peanut; WKB 3 = 60% maize + 30% AYB + 10% peanut; WKB 4 = 50% maize + 40% AYB + 10% peanut.

3.4. In Vivo Glycemic Index (GI) and Blood Glucose Concentration of Rats Fed with Aadun Samples

The blood glucose concentration of Wistar rats fed with *aadun* samples is presented in Figure 5. At time zero (0 min), the rats fed with sample WKB 1 (100% maize) and WKB 3 (60:30:10%; maize:AYB:peanut) showed the highest and least concentrations, respectively. After 15 mins, sample WKB 3 showed stable concentration all through the 2 h period. This might be due to the high level of its chemical compositions and amino acid profile. Meanwhile, sample WKB 2 (70:20:10%; maize:AYB:peanut) had a peak load at 15 min but the peak continually diminished for 120 mins. However, after 60 mins, all the rats fed with the *aadun* samples experienced a drop in blood glucose concentrations when compared to the rats fed with glucose, which showed that the sugars in these *aadun* products were not undergoing rapid or spontaneous digestion over a period of time.

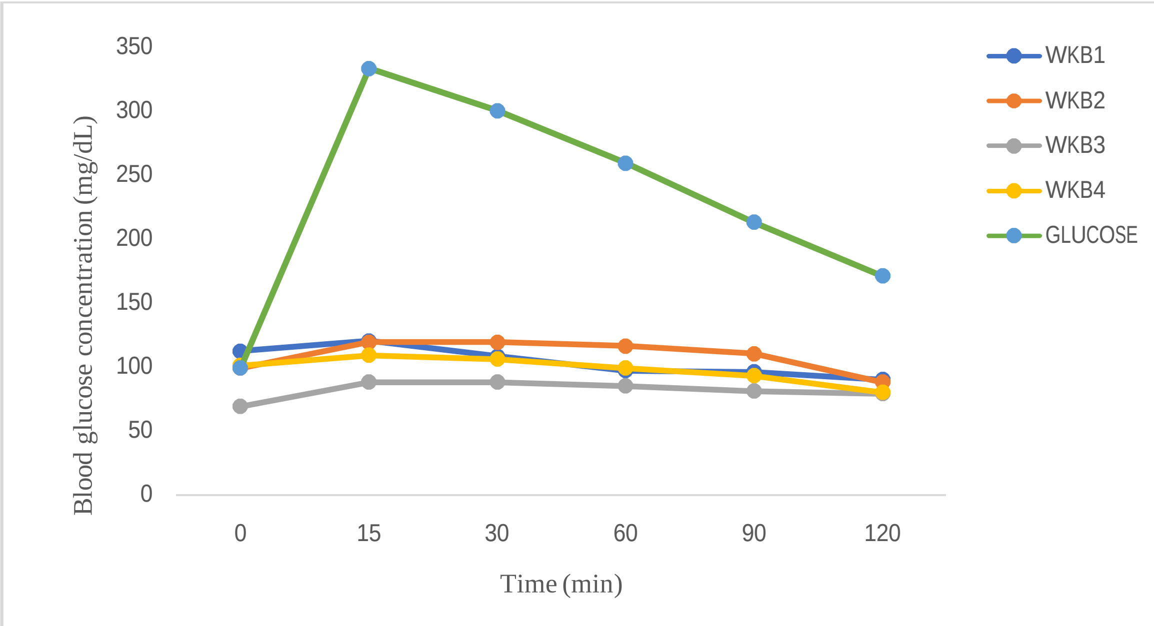

Figure 5. Blood glucose concentration of rats fed with different *aadun* samples.

Key: WKB 1 = 100% maize; WKB 2 = 70% maize + 20% AYB + 10% peanut; WKB 3 = 60% maize + 30% AYB + 10% peanut; WKB 4 = 50% maize + 40% AYB + 10% peanut.

The results presented in Figure 6 show that all the *aadun* samples have low GI (34.17–45.55%) when compared to the <55% regarded as standard values for low GI foods [42]. This undoubtedly shows that the present *aadun* samples could serve as potential food for Type-2 diabetic patients. The GI value of certain foods depends on the amount and composition of carbohydrates, fat, and protein contents of the food as well as its acidity, particle size, and cooking methods [42]. Another study also observed a change in glucose concentrations to a greater degree in capillary blood samples than in veinous blood samples after food consumption. Therefore, veinous blood may be a more relevant indicator of the physiological consequences of low GI foods [43].

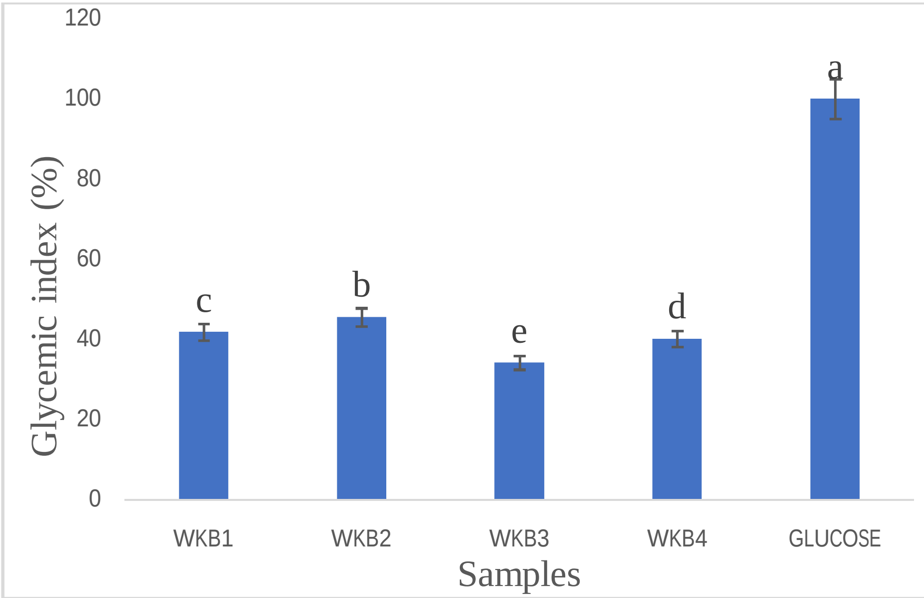

Figure 6. In vivo glycemic index for different *aadun* samples.

Bars (n = 3) with different letters are significantly different ($p < 0.05$). Key: WKB 1 = 100% maize; WKB 2 = 70% maize + 20% AYB + 10% peanut; WKB 3 = 60% maize + 30% AYB + 10% peanut; WKB 4 = 50% maize + 40% AYB + 10% peanut.

3.5. Microbial Loads of Aadun Samples

The microbial loads of *aadun* produced from composite flours of roasted maize, African yam bean,and peanut are shown in Table 4. The composite *aadun* samples generally had low yeast and mold counts of $1.00–1.50 \times 10^{-8}$ cfu; whereas, no yeast or mold growth was observed in WKB 1 (100% maize). This could be due to the concise or thorough roasting process of the starter crops (maize, AYB, and peanut) as well as the viable hygienic conditions during the handling of all production steps. Interestingly, there was no observable growth of the common pathogenic microorganisms like *E. coli.*,Salmonella, general coliforms, and *S. aureus*,which could have been a result of using water and other liquid mediums that were devoid of any fecal contaminations [32]. In contrast, a low count of *S. aureus* (1.30×10^{-8} cfu) was observed in the commercial sample, which could have been due to improper unhygienic production and handling of the commercial products.

Table 4. Microbial loads of *Aadun* samples (Cfu/g).

Samples/Microbes	WKB 1	WKB 2	WKB 3	WKB 4	Commercial Sample
Yeast	NG	1.00×10^{-8}	1.10×10^{-8}	1.20×10^{-8}	1.80×10^{-8}
Mold	NG	1.20×10^{-8}	1.30×10^{-8}	1.50×10^{-8}	2.00×10^{-8}
E. coli.	NG	NG	NG	NG	NG
Salmonella	NG	NG	NG	NG	NG
Coliform	NG	NG	NG	NG	1.10×10^{-8}
S. aureus	NG	NG	NG	NG	1.30×10^{-8}

Key:WKB 1 = 100% maize; WKB 2 = 70% maize + 20% AYB + 10% peanut; WKB 3 = 60% maize + 30% AYB + 10% peanut; WKB 4 = 50% maize + 40% AYB + 10% peanut. NG = No growth.

3.6. Physical Properties of Aadun Samples

The physical parameters of *aadun* produced from the composite flour blends and control are shown in Table 5. The weight values ranged from 4.14 to 5.62 g, with significant highest and least values reported for WKB 4 (50:40:10%; maize:AYB:peanut) and WKB 1 (100% maize), respectively, but with no observable difference in WKB 2 (70:20:10%; maize:AYB:peanut) and WKB 4. The weight of a food product, which is a reflection of its bulkiness level could be attributed to the individual water-holding capacities of the raw materials involved, while its ease of packaging is a function of its low weight [44]. The thickness of the *aadun* ranged from 3.30–3.37 mm with the highest and lowest values reported for WKB 4 and WKB 1, respectively. This is lower than those for the control sample (3.53 mm). This showed that the substitution process did not have a negative effect on the produced *aadun*. The results of the thickness of the aadun reflected no significant difference in samples WKB 1 and WKB 3 (60:30:10%; maize:AYB:peanut), but the difference (p< 0.05) was observed in sample WKB 2 and (p> 0.05) in sample WKB 4. The present result is in agreement with past findings that reported no significant difference in the *aadun* produced from maize-groundnut paste [34]. Although the width of the *aadun* ranged from 32.09–32.64 mm, there was no observable difference in the samples, including the control samples. The spread ratio of the aadun ranged from 9.69 to 9.88 with no significant difference between the samples as well as the control samples.

Table 5. Physical properties of different *aadun* samples.

Samples/Parameters	WKB 1	WKB 2	WKB 3	WKB 4	Commercial Sample
Weight (g)	4.14 ± 0.07 ^{b}	5.62 ± 0.02 ^{a}	5.02 ± 0.02 ^{a}	5.62 ± 0.02 ^{a}	4.77 ± 0.74 ^{b}
Width (mm)	32.09 ± 0.17 ^{a}	32.39 ± 0.06 ^{a}	32.59 ± 0.46 ^{a}	32.64 ± 0.32 ^{a}	32.13 ± 0.91 ^{a}
Thickness (mm)	3.30 ± 0.08 ^{ab}	3.34 ± 0.28 ^{a}	3.30 ± 0.51 ^{ab}	3.37 ± 0.28 ^{a}	3.53 ± 0.47 ^{a}
Spread ratio	9.72 ± 0.04 ^{b}	9.70 ± 0.01 ^{b}	9.88 ± 0.01 ^{a}	9.69 ± 0.01 ^{b}	9.10 ± 0.01 ^{c}

Means (n = 3) with different letters in the row are significantly different (p< 0.05). **Key:** WKB 1 = 100% maize; WKB 2 = 70% maize + 20% AYB + 10% peanut; WKB 3 = 60% maize + 30% AYB + 10% peanut; WKB 4 = 50% maize + 40% AYB + 10% peanut.

3.7. Sensory Attributes of Aadun Samples

The organoleptic properties of *aadun* produced from maize, African yam bean (AYB), and peanut flour are presented in Table 6. Although, the panelists mostly preferred the commercial sample when compared to the experimental formulated samples, the formulated samples WKB 1, WKB 2, and WKB 3 showed no significant difference (p> 0.05) in their color, taste, texture, crispness, appearance, and aroma. This showed that the level of substitution did not have a significantly negative influence on the color, taste, texture, crispness, appearance, and aroma of the *aadun* samples. In contrast, there existed an observable significant difference ($p < 0.05$) in aroma, taste, and appearance of sample WKB 4 when compared to the other samples, which could be due to its high composition (40%) of AYB. Interestingly, all the *aadun* samples were overall accepted by the panelists.

Table 6. Sensory attributes of different *aadun* samples.

SAMPLES	COLOR	TASTE	TEXTURE	CRISPNESS	APPEARANCE	AROMA	OVERALL ACCEPT.
WKB 1	8.50^{b}	8.26^{b}	8.70^{b}	8.95^{a}	8.20^{c}	8.70^{b}	8.90^{a}
WKB 2	8.90^{a}	8.80^{b}	8.85^{ab}	8.90^{a}	8.65^{b}	8.70^{b}	8.70^{b}
WKB 3	8.55^{b}	8.30^{b}	8.60^{b}	8.00^{b}	8.30^{cd}	8.55^{c}	8.45^{c}
WKB 4	7.15^{c}	6.10^{c}	7.15^{c}	7.05^{c}	6.05^{d}	5.65^{d}	7.10^{d}
Commercial product	8.92^{a}	8.95^{a}	8.91^{a}	8.94^{a}	8.78^{a}	8.89^{a}	8.95^{a}

Means (n = 50) with different letters in the column are significantly different ($p < 0.05$). **Key:** WKB 1 = 100% maize; WKB 2 = 70% maize + 20% AYB + 10% peanut; WKB 3 = 60% maize + 30% AYB + 10% peanut; WKB 4 = 50% maize + 40% AYB + 10% peanut.

4. CONCLUSIONS

The study was carried out to compare *aadun* made from flour blends containing maize, African yam bean (AYB), and peanut with those made from 100% maize flour. The blended *aadun* could serve as a nutritious food or snack and help to redress the problem of protein energy malnutrition due to the findings from the present study. *Aadun* from the composite flour blends of maize, African yam beans, and peanut had high protein (~14%), BV (~90%), and HAA (40%) when compared to those from 100% maize (11, 72, and 30%, respectively). The obvious observation could be due to the incorporation of AYB and peanuts into the composite flour. The study further showed that the level of substitution of maize flour with flour blends from locally grown underutilized crops has a great influence on the nutritional quality of the *aadun* being produced. The low GI (~46%) and increased antioxidant (~80%) of the composite *aadun* contributed to its enhanced blood glucose-reducing (anti-diabetic) properties. The resultant *aadun* samples were devoid of microbial loads (no or low growths) and also, overall, compared well to the consumer attributes of the commercial samples. The physical properties of the aadun revealed no significant changes in the appearance of the snacks from the maize or formulated flour blends. Sample WKB 3 (60:30:10%; maize:AYB:peanut) had better nutritional, amino acid profiles, and antioxidant and anti-diabetic properties when compared to other samples. Sample WKB 4 (50:40:10%; maize:AYB:peanut) was rated least acceptable by the consumers when compared with the other formulated samples, which might be a result of the high amount of AYB (40%) in the sample WKB 4.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

Ethical Approval: The study protocol was approved and ethic clearance was given by the Ethical Committee for Laboratory Research of the School of Agriculture and Agricultural Technology, Federal University of Technology, Akure, Nigeria (FUTA/SAAT/2022/033).

DATA AVAILABILITY STATEMENT: Data are available upon request by contacting the authors.

ABBREVIATIONS

DPPH = 2, 2-diphenyl-1-picrylhydrazyl (DPPH); FRAP = Ferric reducing antioxidant power; GAE = Gallic acid equivalents; HCl = Hydrochloric acid; HPLC = High-performance liquid chromatography; TCA = Trichloroacetic acid.

Disclaimer (Artificial intelligence)

Option 1:

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

Option 2:

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc have been used during writing or editing of manuscripts. This explanation will include the name, version, model, and source of the generative AI technology and as well as all input prompts provided to the generative AI technology

Details of the AI usage are given below:

1.

2.

3.

REFERENCES

1. Hammer MJ. Hyperglycemia and cancer: a state-of-the-science review. Number 4/July 2019. 2019 Jul 1;46(4):459-72.
2. Yari Z, Behrouz V, Zand H, Pourvali K. New insight into diabetes management: from glycemic index to dietary insulin index. Current diabetes reviews. 2020 May 1;16(4):293-300.
3. Alharbi KS, Alenezi SK, Gupta G. Pathophysiology and pathogenesis of inflammation. InRecent Developments in Anti-Inflammatory Therapy 2023 Jan 1 (pp. 1-9). Academic Press.

4. Zhang J, Chen L, Delzell E, Muntner P, Hillegass WB, Safford MM, et al. The association between inflammatory markers, serum lipids and the risk of cardiovascular events in patients with rheumatoid arthritis. Annals of the rheumatic diseases. 2014 Jul 1;73(7):1301-8.
5. Makinde OM, Sulyok M, Adeleke RA, Krska R, Ezekiel CN. Bacteriological Quality and Biotoxin Profile of Ready-to-Eat Foods Vended in Lagos, Nigeria. Foods. 2023 Mar 13;12(6):1224.
6. Amanyunose AA, Olosunde OO, Adedeji TO, Abiodun OA. Effect of Roasted Soybean Flour Substitution on the Chemical and Sensory Properties of Maize Flour Snack (Aadun). Asian Food Science Journal. 2021 Feb 12;20(1):72-7.
7. Akinola SA, Enujiugha VN. Physicochemical and sensory qualities of “aadun” a maize based snack supplemented with defatted African oil bean seed flour. Applied Tropical Agriculture. 2017;22(2):188-96.
8. Idowu MA, Atolagbe YM, Henshaw FO, Akpan I, Oduse K. Quality assessment and safety of street vended „aadun‟(a maize-based indigenous snack) from selected states in Nigeria. African Journal of Food Science. 2012 Oct 15;6(19):474-82.
9. Wang B, Lin Z, Li X, Zhao Y, Zhao B, Wu G, et al. Genome-wide selection and genetic improvement during modern maize breeding. Nature Genetics. 2020 Jun;52(6):565-71.
10. Zhan T, Han Y, Tang C, Zhao Q, Sun D, Li Y, et al.. Metabolism and biological activity of α□tocopherol derived from vitamin E□enriched transgenic maize in broilers. Journal of the Science of Food and Agriculture. 2020 Aug 30;100(11):4319-28.
11. Nuss ET, Tanumihardjo SA. Maize: a paramount staple crop in the context of global nutrition. Comprehensive reviews in food science and food safety. 2010 Jul;9(4):417-36.
12. Ajibola CF, Fashakin JB, Fagbemi TN, Aluko RE. Effect of peptide size on antioxidant properties of African yam bean seed (Sphenostylis stenocarpa) protein hydrolysate fractions. International journal of molecular sciences. 2011 Oct 11;12(10):6685-702.
13. Oludumila OR, Enujiugha VN. Physicochemical and rheological properties of complementary diet from blends of maize, African yam bean and pigeon pea flour. Sci J Food Sci Nutr. 2017;3:5-11.
14. Suchoszek-Łukaniuk K, Jaromin A, Korycińska M, Kozubek A. Health benefits of peanut (Arachis hypogaea L.) seeds and peanut oil consumption. InNuts and seeds in health and disease prevention 2011 Jan 1 (pp. 873-880). Academic Press.
15. Campos-Mondragón MG, De La Barca AC, Durán-Prado A, Campos-Reyes LC, Oliart-Ros RM, Ortega-García J, Medina-Juárez LA, Angulo O. Nutritional composition of new peanut (Arachis hypogaea L.) cultivars. Grasas y aceites. 2009 Jun 30;60(2):161-7.
16. Shittu TA, Raji AO, Sanni LO. Bread from composite cassava-wheat flour: I. Effect of baking time and temperature on some physical properties of bread loaf. Food research international. 2007 Mar 1;40(2):280-90.
17. Adeyanju BE, Enujiugha VN, Bolade MK. Effects of addition of kidney bean (Phaseolus vulgaris) and alligator pepper (Aframomum melegueta) on some properties of ‘aadun’(a popular local maize snack). Journal of Sustainable Technology. 2016 Apr;7(1):45-58.

18. Noorfarahzilah M, Lee JS, Sharifudin MS, Mohd Fadzelly AB, Hasmadi M. Applications of composite flour in development of food products. International Food Research Journal. 2014 Dec 1;21(6).
19. Hugo LF, Rooney LW, Taylor JR. Malted sorghum as a functional ingredient in composite bread. Cereal chemistry. 2000 Jul;77(4):428-32.
20. Mamat H, Matanjun P, Ibrahim S, Md. Amin SF, Abdul Hamid M, Rameli AS. The effect of seaweed composite flour on the textural properties of dough and bread. Journal of applied phycology. 2014 Apr;26:1057-62.
21. Malomo SA, Eleyinmi AF, Fashakin JB. Chemical composition, rheological properties and bread making potentials of composite flours from breadfruit, breadnut and wheat. African Journal of Food Science. 2011 Jul;5(7):400-10.
22. Kaushal P, Kumar V, Sharma HK. Comparative study of physicochemical, functional, antinutritional and pasting properties of taro (Colocasia esculenta), rice (Oryza sativa) flour, pigeonpea (Cajanus cajan) flour and their blends. LWT-Food Science and Technology. 2012 Sep 1;48(1):59-68.
23. Arise AK, Malomo SA, Awaw AA, Arise RO. Quality attributes and consumer acceptability of custard supplemented with bambara groundnut protein isolates. Applied Food Research. 2022 Jun 1;2(1):100056.
24. 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 Jun 1;2(1):100097.
25. IJAROTIMI OS, OLUWAJUYITAN TD, OGUNMOLA GT. Nutritional, functional and sensory properties of gluten-free composite flour produced from plantain (Musa AAB), tigernut tubers (Cyperus esculentus) and defatted soybean cake (Glycine max). Croatian journal of food science and technology. 2019 May 30;11(1):1131-251.
26. Akinbode BA, Malomo SA, Asasile II. In vitro antioxidant, anti-inflammatory and in vivo anti-hyperglycemia potentials of cookies made from sorghum, orange-flesh-sweet-potato and mushroom protein isolate flour blends fed to Wistar rats. Food Chemistry Advances. 2023 Oct 1;2:100263.
27. Arise AK, Malomo SA, Acho MA, Ajao-Azeez ND, Arise RO. In vivo anti-diabetic activity, physicochemical and sensory properties of Kunu enriched with African walnut. Food Chemistry Advances. 2023 Oct 1;2:100315.
28. Onabanjo OO, Ighere DA. Nutritional, functional and sensory properties of biscuit produced from wheat-sweet potato composite. Journal of Food Technology Research. 2014;1(2):111-21.
29. Sodipo MA, Fashakin JB. Physico-chemical properties of a complementary diet prepared from germinated maize, cowpea and pigeon pea. Journal of Food, Agriculture and Environment. 2011 Jul 1;9(3-4):23-5.
30. Sani M, Raji AO, Raji MO. Evaluation of weaning food blends from modified millet and groundnut cake flour. Technoscience Journal for Community Development in Africa. 2020 Oct 4;1(1):33-40.
31. Horwitz W, Latimer GW. Official methods of analysis of AOAC International. Gaithersburg: AOAC international; 2000.
32. Malomo SA, Niwachukwu ID, Girgih AT, Idowu AO, Aluka RE, Fagbemi TN. Antioxidant and renin-angiotensin system inhibitory properties of cashew nut and fluted-

pumpkin protein hydrolysates. Polish Journal of Food and Nutrition Sciences. 2020;70(3).
33. Oluwajuyitan TD, Ijarotimi OS. Nutritional, antioxidant, glycaemic index and Antihyperglycaemic properties of improved traditional plantain-based (Musa AAB) dough meal enriched with tigernut (Cyperus esculentus) and defatted soybean (Glycine max) flour for diabetic patients. Heliyon. 2019 Apr 1;5(4).
34. Amakoromo ER, Innocent-Adiele HC, Njoku HO. Microbiological quality of a yoghurt-like product from african yam bean. National Science. 2012;10(6):6-9.
35. 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):82-9.
36. Orhevba BA, Atteh BO. Effect of Different Packaging Materials on Some Properties of Enhanced Aadun (Maize Meal Snack).
37. Olaiya AB, Adelola OB. 3PROXIMATE AND SENSORY ASSESSMENT OF AADUN PRODUCED FROM MAIZE AND GROUNDNUT PASTE. LOCAL ORGANISING COMMITTEE (LOC). 2022:15.
38. George TT, Obilana AO, Oyeyinka SA. The prospects of African yam bean: past and future importance. Heliyon. 2020 Nov 1;6(11).
39. Roy S, Hazra B, Mandal N, Chaudhuri TK. Assessment of the antioxidant and free radical scavenging activities of methanolic extract of Diplazium esculentum. International Journal of Food Properties. 2013 Aug 18;16(6):1351-70.
40. Gervasi T, Oliveri F, Gottuso V, Squadrito M, Bartolomeo G, Cicero N, Dugo G. Nero d'Avola and Perricone cultivars: Determination of polyphenols, flavonoids and anthocyanins in grapes and wines. Natural Product Research. 2016 Oct 17;30(20):2329-37.
41. Gordon MH. Significance of dietary antioxidants for health. International Journal of Molecular Sciences. 2011 Dec 23;13(1):173-9.
42. Pinheiro J, Gonçalves EM, Ganhão R. Potential Use of Aqueous Extracts of Kombu Seaweed in Cream Cracker Formulation. InSustainable Innovation in Food Product Design 2021 Jun 1 (pp. 171-186). Cham: Springer International Publishing.
43. Moktan B, Roy A, Sarkar PK. Antioxidant activities of cereal-legume mixed batters as influenced by process parameters during preparation of dhokla and idli, traditional steamed pancakes. International journal of food sciences and nutrition. 2011 Jun 1;62(4):360-9.
44. Eleazu CO. The concept of low glycemic index and glycemic load foods as panacea for type 2 diabetes mellitus; prospects, challenges and solutions. African health sciences. 2016 Jul 1;16(2):468-79.
45. Foster-Powell K, Holt SH, Brand-Miller JC. International table of glycemic index and glycemic load values: 2002. The American journal of clinical nutrition. 2002 Jul 1;76(1):5-6.
46. Bello FA, Ntukidem OJ, Oladeji BS. Assessment of chemical compositions, physical and sensory properties of biscuits produced from yellow yam, unripe plantain and pumpkin seed flour blends. Int. J. Food Sci. Nutr. Eng. 2018;8(5):119-26.
47. Aluko, Babatunde Olaoluwa, Olufemi Olugbenga Awolu, and Sekinat Olayemi Bamidele. 2024. "Evaluation of the Nutritional, Antioxidant and Physiochemical Properties of Rice Flour Fortified With the Nigeria Native 'IGBEMO' Rice Bran Flour". Asian Food Science Journal 23 (1):12-33. https://doi.org/10.9734/afsj/2024/v23i1692.

48. Nishu, Monika Sood, Julie D. Bandral, Neeraj Gupta, and Duwa. 2024. "A Study on Storage Stability of Brown Rice-Based Instant Khichdi Mix". Archives of Current Research International 24 (4):30-38. https://doi.org/10.9734/acri/2024/v24i4657.
49. Chaudhari AK, Singh VK, Das S, Prasad J, Dwivedy AK, Dubey NK. Improvement of in vitro and in situ antifungal, AFB1 inhibitory and antioxidant activity of Origanum majorana L. essential oil through nanoemulsion and recommending as novel food preservative. Food and Chemical Toxicology. 2020 Sep 1;143:111536.