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Biochemical parameters in blood serum of Wistar rats fed with biscuits formulated from graded substitution of wheat flour with Bambara groundnut flour and concentrate

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Abstract

Aim: Bambara groundnut is a leguminous plant rich in protein and fiber, generally low in fat and micro nutrients, but relatively rich in iron and zinc. This study aimed at evaluating the impact of graded substitution of wheat flour with Bambara groundnut flour and concentrate in the production of biscuits.

Methods: Biscuits were produced from WBF1 (85% wheat flour and 15% Bambara flour), WBF2 (76% wheat flour and 24% Bambara flour), WBF3 (53% wheat flour and 47% Bambara flour), WBC1 (85% wheat flour and 15% Bambara concentrate), WBC2 (76% wheat flour and 24% Bambara concentrate) and WBC3 (53% wheat flour and 47% Bambara concentrate), based on optimized mixture design of response surface methodology. The animal (albino rats of the Wistar strain) experiments were carried out according to the animal protection law. They were divided into eight groups of five rats each and were fed with the formulated diet for 21 days after seven days of acclimatization. The proximate and biochemical parameters of the biscuits were determined.

Results: There was a significant increase in the crude protein content of the biscuit ranging from 15.30% to 20.10% for Bambara groundnut flour biscuit, and 26.20% to 36.20% for Bambara groundnut concentrate biscuit, as compared to wheat flour biscuit (10.37%). The biochemical markers for all the animal groups were significantly different ($p < 0.05$) and generally improved with Bambara nut substitution. The total protein ranged between 4.45–6.42 g/dL and urea (10.57–19.33 mg/dL), falling within the standard ranges. The aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ratios were less than 1 for all the samples. The cholesterol, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) levels were generally low.

Conclusions: From this study, Bambara groundnut increased the protein content of wheat biscuit, and the consumption of this biscuit might not pose any acute danger to the liver and kidney functioning mechanisms.

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Keywords

Vigna subterranea, composite flour, protein concentrate, serum biochemistry, liver function, kidney function

Introduction

In developing countries such as Nigeria, there is high under nutrition which manifests mainly as protein-energy malnutrition (PEM) and micronutrient deficiencies. PEM is a spectrum of illnesses arising from the deficiency/imbalance of protein and energy, which is commonly associated with stunted growth or wasting [1]. It is often seen in infants and young children, lactating women, and during periods of famine or emergencies. Extreme cases of PEM lead to kwashiorkor, marasmus, or marasmic-kwashiorkor [2, 3]. Malnutrition, which has been discovered to be one of the major dietary problems in Nigeria, cutting across all ages, has led to high consumers' interest in functional foods due to their potential health benefits in the prevention of some ailments or deficiencies such as stunted growth, underweight, cardiovascular diseases, inflammation, and obesity [4, 5]. Functional foods are mostly made up of composite flour from underutilized cereals, legumes, and root crops such as Bambara groundnut. Composite flour is developed to cater for specific nutrient composition and functional characteristics deficient in single-matrix flours like wheat flour, which is the most common flour used in baked goods like biscuits, bread, dough meals, etc., widely consumed by all age groups. In the production of cookies, Bambara groundnut has been used as a substitute flour with high sensory acceptability and nutritional quality [6].

Bambara groundnut (*Vigna subterranea* L.) is an ancient African pulse plant, which ranks as the third most significant legume when it comes to consumption and socioeconomic impact in semi-arid regions in Africa after peanut (*Arachis hypogaea*) and cowpea (*Vigna unguiculata*). It is an incongruity that an ancient African crop which provides a nearly complete nutritional profile, is easy to cultivate and requires minimal soil inputs, remains underutilized in its native regions [7]. Bambara groundnut is reported to be high in protein (20–26% crude protein), an important macronutrient for humans [8]. In this light, Bambara groundnut is considered a balanced food compared to most legumes, considering its high content of protein and iron, and its amino acid composition includes lysine and methionine [9]. Moreover, it has been reported that Bambara groundnut contains 63% carbohydrates and 18% oil, with fatty acids dominated by linoleic, palmitic, and linolenic acids [8]. Due to the high price of animal protein products, high value has been placed on leguminous plants as protein-rich substitutes for mitigating widespread malnutrition in developing countries, which are cheaper and readily available. Also, the use of composite flour is meant to mitigate gluten-related celiac disease and to have products that are rich in protein and essential micronutrients in developing countries [5].

Wheat is the most common cereal flour used in the confectionery industries owing to its content of gluten, a protein complex (containing principal proteins, α-gliadin and α-glutenin) which makes it act like a stretchy or elastic material. Wheat grain is made up of 13–17% bran, 2–3% germ, and 81–84% endosperm. The key elements of the endosperm are starch (70%), proteins (6–20%), moisture (14%), mineral (ash) (0.4%), sugar (1%), lipid (1.0%), and others (2.1%) [10]. It has been discovered that Bambara groundnut is a reliable source of protein, having some essential amino acids such as lysine, isoleucine, leucine, methionine, phenylalanine, valine, threonine, and fibre, which is able to reduce protein energy malnutrition [11]. In recent years, structured meals have been replaced with ready-to-eat (R-T-E) foods and on-the-go snacks despite the increasing awareness promoting healthy food options and functional foods. This has led to innovative product development to incorporate functional components and compounds with high nutritional and health-promoting characteristics into these R-T-E foods and everyday consumer snacks. Thus, due to limited data on the biochemical effects of Bambara-based diets on animal models and the high consumption of biscuits in the consumer market, this study evaluated the biochemical indices in rats fed with biscuits produced from a mixture of flour/concentrate from Bambara groundnut and wheat flour. This was with a view to determining the nutritional efficacy of the compounded diet on consumer health.

Materials and methods

Sources of materials and experimental animals

The raw materials, wheat and Bambara groundnut seed, were purchased at Owena Market along Akure–Ilesha Road, Osun State, while the sugar, milk, flavor, eggs, and fat were purchased at Erekesan Market, Akure, Ondo State. Other materials and equipment such as bowls, sieve, cabinet dryer, and hammer mill were obtained from the Food Processing Laboratory of the Department of Food Science and Technology, FUTA. Forty (40) healthy weanling male Albino Wistar rats used in this study were gotten from the Department of Biochemistry, Federal University of Technology, FUTA, Akure, Nigeria. The study was approved by the School of Agriculture and Agricultural Technology, Federal University of Technology, Akure, Ethical Committee under the ethical number FUTA/SAAT/2024/022, in compliance with the Guide for the Care and Use of Laboratory Animals. Reagents and chemicals used were obtained from a renowned chemical store and were of analytical grade.

Preparation of samples

Production of wheat flour

The wheat grains were sorted to remove extraneous materials and it was milled using the attrition milling machine (ADDIS A11, NIGERIA) with a motor type YL 90L-4. Then it was sieved to have a fine flour. The wheat flour was stored in a plastic bag before production.

Production of Bambara flour

Bambara groundnut flour was produced according to the method of Arise et al. [12] with slight modifications. Bambara groundnuts were sorted and soaked in water for 72 hours at tropical ambient temperature (28 ± 2°C), with daily changes of water; they were then decorticated and washed, dried in an air oven at 32°C for 48 hours and milled using the attrition mill (ADDIS A11, NIGERIA) with a motor type YL 90L-4 and sieved for uniform particle size using sieve pore sizes of 4.75 mm to 75 µm. The flour was then packaged in a high-density polyethylene plastic container and kept at 4°C prior to further use.

Production of Bambara protein concentrate flour

The protein concentrates from Bambara groundnut flour were obtained following the alkaline wet extraction method outlined by Kiin-Kabari and Giami [13]. A 50 g sample of flour was measured and dispersed in 300 mL of 0.04 M sodium hydroxide (NaOH), after which the mixture was continuously stirred at room temperature (28 ± 2°C) for one hour using a mechanical shaker (MXBAOHENG MPL-10). The pH of the resulting slurry was then adjusted to 10.8 with 1.0 M NaOH before centrifugation at 3,500 g for 25 minutes, separating it into a residue and a supernatant. To enhance protein yield, the residue was re-suspended in the alkaline solution, and the extraction process was repeated. The collected extracts were then acidified to pH 4.5 using 1 M hydrochloric acid (HCl) to facilitate protein precipitation. The precipitated proteins were isolated through centrifugation for 15 minutes, washed twice, and neutralized to pH 7.0 using 1 M NaOH. Finally, the protein concentrate was dried using hot air for 18 hours and stored at 4°C for subsequent use.

Preparation of the flour blends

The wheat flour and the Bambara groundnut flour were formulated as shown in Table 1.

Table 1. Formulation and mixing ratios of the composite flour.

Formulation and mixing ratios		
Samples	Wheat flour (%)	Bambara flour (%)
WBF1	85	15
WBF2	76	24
WBF3	53	47

Table 1. Formulation and mixing ratios of the composite flour. (*continued*)

Formulation and mixing ratios		
Samples	Wheat flour (%)	Bambara protein concentrate flour (%)
WBC1	85	15
WBC2	76	24
WBC3	53	47

Production of Bambara-wheat biscuit

The biscuit was prepared according to Adegbanke et al. [9] with slight modifications. The composite flour was weighed according to the specified ratio for each sample. The other ingredients were weighed and added to the flour, such as milk, baking powder, salt, sugar, margarine, flavor, and nut-meg. This was all mixed together for about 5 minutes before adding water. After the dough was formed, it was properly kneaded before it was later rolled out and cut into shapes. The shaped dough was arranged on the tray and placed into the pre-heated oven. It was baked at 180–200°C for 15 minutes and it was allowed to turn golden brown. It is allowed to cool down before it is stored inside a polyethylene bag.

Animal experimental design

Weanling male Albino Wistar rats that were used for this experiment were procured from the Department of Biochemistry animal house, Federal University of Technology, Akure, Nigeria and were maintained at 25°C on a 12-h light/dark cycle and granted free access to food and water. They were acclimatized under these conditions for one (1) week before beginning the experiment. The rats were separated into eight groups in accordance with their body weight, comprising five animals each, as reported in Table 2. The animals had unrestricted access to food and clean water at every hour of the day (feeding was *ad libitum*, according to standard protocols for animal experiments).

Table 2. The grouping of the rats according to their diets.

Groups	Diets
Group 1	Pure Bliss® biscuit (positive control with 72.1 g/100 g carbohydrate, 10.2 g/100 g protein, 461 kcal energy)/casein (1:2 ratio)
Group 2	WBC1 (85% wheat and 15% Bambara groundnut concentrate)
Group 3	WBC2 (76% wheat and 24% Bambara groundnut concentrate)
Group 4	WBC3 (53% wheat and 47% Bambara groundnut concentrate)
Group 5	WBF1 (85% wheat and 15% Bambara groundnut flour)
Group 6	WBF2 (76% wheat and 24% Bambara groundnut flour)
Group 7	WBF3 (53% wheat and 47% Bambara groundnut flour)
Group 8	Basal diets (negative control)

After 21 days of treatments, carbon dioxide asphyxiation and subsequent cervical dislocation were used to immobilize them before the animals were sacrificed; this was done to minimize struggle and preserve blood flow. The blood was collected using a syringe through cardiac puncture and transferred into non-heparinized tubes and placed on ice. It was then kept inside the refrigerator (at 4°C) for further analyses.

Analyses

Analyses were carried out to determine the biochemical parameters in the experimental rats and the proximate composition of the biscuit.

Determination of proximate composition of the Bambara-wheat biscuit

It involves the determination of the moisture content, ash content, crude protein, crude fat, and carbohydrate (by difference). The proximate composition was determined in accordance with the standard

method of AOAC [14]. Fat analysis was determined using a Soxhlet extractor, crude protein was determined using the Kjeldahl method, moisture was also determined using the air-oven method, and ash was determined using a muffle furnace. The carbohydrate was calculated by difference.

Biochemical determination

The biochemical parameters were assessed following the procedures outlined by Lala et al. [15]. Initially, the blood sample was centrifuged (3,000 rpm, 10 minutes, 4°C) to separate the serum. The obtained serum was subsequently utilized for liver function analysis by measuring the enzymatic activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP). Additionally, kidney function was measured based on serum concentrations of urea and creatinine. The tests were evaluated using disposable kits gotten from Lab Test Diagnostica S.A. (Lagoa Santa, Minas Gerais, Brazil). The lipids and lipoproteins of the blood samples were also determined.

Statistical analysis

The analyses were carried out in triplicate, and the results were subjected to statistical analysis using the statistical package for social statistics (IBM version. 20.0, SPSS Inc., Quarry Bay, Hong Kong) and presented as means (SEM). The mean and standard deviation of the triplicate analysis of the samples were calculated. One-way analysis of variance (ANOVA) was conducted to assess significant differences among the means of the proximate composition, and mean separation was carried out using Duncan's Multiple Range Test (DMRT). Values of $p < 0.05$ were considered statistically significant, as described in a previous study [11].

Results

The proximate composition of the biscuits

The proximate analyses generated the results of protein, carbohydrate, crude fibre, fat, moisture, total ash, and energy contents as presented in Table 3. The results of the proximate composition indicate high protein levels in the various samples except for the 100% wheat flour biscuit. The values ranged from 10.37 ± 0.07% in sample WF to 36.20 ± 0.03% in sample WBC3. Sample WBC2 had the second largest protein content with value 31.30% and WBC1 with value 26.20%. Other samples' (WBF1, WBF2 and WBF3) crude protein values were considerably high too, with values of 15.30 ± 0.33%, 18.60 ± 0.12% and 20.10 ± 0.54%, respectively. The results of the fat showed that it ranged from 4.69 ± 0.02% in sample WBC3 to 7.80 ± 0.25% in sample WBF1. The result of the crude fiber showed that it ranged from 1.30 ± 0.01% in sample WBF1 to 2.50 ± 0.20% in sample WBC2. The moisture content ranged from 5.00% in sample WBC3 to 8.69% in sample WF. The moisture content is lower than that of the 100% wheat flour. The carbohydrate content ranged from 49.50 ± 0.03% in sample WBC3 to 71.03 ± 0.41% in sample WF. Results obtained from statistical analysis showed that there is a notable difference between the samples at $p < 0.05$. The available carbohydrates in the biscuit samples incorporated with Bambara groundnut flour and protein concentrate were lower than those in the control sample.

Table 3. Proximate composition (%) of the biscuits made from the composite flours.

Samples	Moisture content%	Crude protein%	Crude fat%	Total ash%	Crude fibre%	Carbohydrate%	Energy (kcal/100 g)
WBF1	7.60 ± 0.53^{a}	15.30 ± 0.33^{f}	7.80 ± 0.25^{a}	1.00 ± 0.28^{g}	1.30 ± 0.01^{f}	67.00 ± 0.72^{b}	399.20 ± 4.20^{a}
WBF2	7.20 ± 0.07^{c}	18.60 ± 0.12^{e}	7.20 ± 0.41^{b}	1.40 ± 0.07^{f}	1.70 ± 0.54^{e}	63.90 ± 0.54^{c}	394.80 ± 3.20^{ab}
WBF3	6.70 ± 0.34^{d}	20.10 ± 0.54^{d}	6.40 ± 0.07^{c}	1.70 ± 0.21^{d}	2.00 ± 0.54^{c}	63.10 ± 0.02^{d}	390.40 ± 1.20^{b}
WBC1	5.30 ± 0.45^{f}	26.20 ± 0.03^{c}	6.30 ± 0.74^{d}	2.50 ± 0.05^{c}	2.20 ± 0.07^{b}	57.50 ± 0.81^{e}	391.50 ± 1.20^{b}
WBC2	5.70 ± 0.12^{e}	31.30 ± 0.01^{b}	5.90 ± 0.05^{e}	2.60 ± 0.03^{b}	2.50 ± 0.20^{a}	52.00 ± 0.04^{f}	386.30 ± 0.90^{bc}

Table 3. Proximate composition (%) of the biscuits made from the composite flours. (*continued*)

Samples	Moisture content%	Crude protein%	Crude fat%	Total ash%	Crude fibre%	Carbohydrate%	Energy (kcal/100 g)
WBC3	5.00 ± 0.02^{g}	36.20 ± 0.03^{a}	4.69 ± 0.02^{f}	2.80 ± 0.01^{a}	1.90 ± 0.03^{cd}	49.50 ± 0.03^{g}	393.21 ± 1.80^{b}
WF	8.69 ± 0.02^{b}	10.37 ± 0.07^{f}	6.22 ± 0.02^{c}	1.58 ± 0.03^{e}	2.11 ± 0.01^{c}	71.03 ± 0.41^{a}	381.60 ± 1.30^{c}

Means (SEM) with different alphabetical superscripts in the same column are significantly different at $p < 0.05$. WBF1: 85% wheat and 15% of Bambara groundnut flour, WBF2: 76% wheat and 24% of Bambara groundnut flour, WBF3: 53% wheat and 47% of Bambara groundnut flour, WBC1: 85 % wheat and 15% of Bambara groundnut concentrate, WBC2: 76% wheat and 24% of Bambara groundnut concentrate, WBC3: 53% wheat and 47% of Bambara groundnut concentrate, WF: 100 % wheat flour.

The growth rate of the experimental animals fed with biscuits made from the Bambara composite flours

Table 4 shows the rate of increment in length of the animals fed with the biscuits. There is a significant increase in the length of the animals fed with the biscuits. The casein group has the highest growth rate when compared with other groups. It increased from 13.20 to 18.50 cm. Also, WBC2 and WBC3 have a great increment next to the casein sample, with values increasing from 12.90 to 16.00 cm and 13.10 to 16.80 cm, respectively. This might be due to the high protein content in the formulated diet.

Table 4. Length of the experimental animals fed with biscuits made from the composite flours.

Experimental days	Casein (cm)	WBC1 (cm)	WBC2 (cm)	WBC3 (cm)	WBF1 (cm)	WBF2 (cm)	WBF3 (cm)	Basal (cm)
0	13.20	12.10	12.90	13.10	11.80	11.70	13.40	11.10
4	14.00	12.40	12.40	14.10	12.00	11.80	12.80	10.60
8	15.00	12.80	13.30	15.10	12.40	12.40	12.70	10.80
12	15.70	12.50	13.30	15.10	12.40	12.30	13.30	10.60
16	16.20	13.00	14.00	15.80	12.50	12.30	13.10	10.50
20	17.20	12.90	15.40	15.80	12.50	12.10	13.00	10.00
24	17.70	13.20	15.30	16.30	12.50	12.00	12.90	9.50
28	18.50	14.00	16.00	16.80	12.50	11.90	12.70	9.40

WBF1: 85% wheat and 15% of Bambara groundnut flour, WBF2: 76% wheat and 24% of Bambara groundnut flour, WBF3: 53% wheat and 47% of Bambara groundnut flour, WBC1: 85% wheat and 15% of Bambara groundnut concentrate, WBC2: 76% wheat and 24% of Bambara groundnut concentrate, WBC3: 53% wheat and 47% of Bambara groundnut concentrate.

Table 5 shows the weight gained by the experimental animals as the days progressed. There was an increase in the weight of animals across all the groups except for that of the Basal diet, which has no significant amount of nutrients. Casein, WBC2, and WBC3 showed the highest growth rate of 55.00–143.00 g, 55.00–101.00 g, and 54.00–128.00 g, respectively.

Table 5. Weight of the experimental animals fed with biscuits made from the composite flours.

Experimental days	Casein (g)	WBC1 (g)	WBC2 (g)	WBC3 (g)	WBF1 (g)	WBF2 (g)	WBF3 (g)	Basal (g)
0	55.00	55.00	55.00	54.00	56.00	42.00	56.00	37.00
4	65.00	56.00	60.00	58.00	58.00	45.00	57.00	35.00
8	90.00	60.00	75.00	88.00	60.00	46.00	59.00	32.00
12	105.00	62.00	75.00	106.00	61.00	50.00	60.00	31.00
16	125.00	68.00	86.00	108.00	66.00	52.00	65.00	27.00
20	130.00	68.00	94.00	114.00	76.00	53.00	76.00	24.00
24	137.00	80.00	98.00	127.00	81.00	53.00	81.00	23.00
28	143.00	80.00	101.00	128.00	82.00	54.00	81.00	22.00

WBF1: 85 % wheat and 15% of Bambara groundnut flour, WBF2: 76% wheat and 24% of Bambara groundnut flour, WBF3: 53% wheat and 47% of Bambara groundnut flour, WBC1: 85 % wheat and 15% of Bambara groundnut concentrate, WBC2: 76% wheat and 24% of Bambara groundnut concentrate, WBC3: 53% wheat and 47% of Bambara groundnut concentrate.

Biochemical parameters in the serum of Wistar rats

The effect of biscuits made from wheat, Bambara groundnut concentrate, and Bambara protein flour on the serum biochemical values of male Albino Wistar rats is shown in Tables 6, 7 and 8. The tables presented results for ALT, ALP, AST, total protein, albumin, globulin, creatinine, urea, cholesterol, triglyceride, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) that were significantly different among treatment groups ($p < 0.05$).

Table 6. Kidney function biomarkers and protein profile of the blood serum of rats fed with the formulated biscuits.

Samples	Creatinine (mg/dL)	Urea (mg/dL)	Protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)
Basal	2.33 ± 0.05^{a}	29.88 ± 1.97^{a}	1.93 ± 0.09^{h}	0.66 ± 0.05^{h}	1.27 ± 0.51^{f}
WBF1	2.15 ± 0.06^{a}	19.33 ± 0.64^{b}	4.57 ± 0.07^{e}	2.92 ± 0.05^{g}	2.65 ± 0.07^{a}
WBF2	2.12 ± 0.02^{a}	18.25 ± 1.83^{b}	4.48 ± 0.08^{f}	3.02 ± 0.04^{f}	1.46 ± 0.28^{e}
WBF3	1.44 ± 0.04^{b}	15.16 ± 1.16^{c}	4.45 ± 0.01^{g}	3.12 ± 0.09^{e}	1.88 ± 0.99^{g}
WBC1	1.34 ± 0.07^{c}	12.45 ± 0.73^{d}	5.81 ± 0.04^{c}	3.20 ± 0.07^{d}	2.61 ± 0.28^{a}
WBC2	1.24 ± 0.04^{d}	10.79 ± 2.90^{e}	5.93 ± 0.03^{b}	3.66 ± 0.02^{c}	2.27 ± 0.72^{c}
WBC3	0.98 ± 0.01^{f}	10.57 ± 1.72^{e}	6.42 ± 0.02^{a}	3.92 ± 0.07^{a}	2.50 ± 0.80^{b}
Casein	1.14 ± 0.03^{e}	11.00 ± 0.96^{d}	5.63 ± 0.06^{d}	3.78 ± 0.04^{b}	1.85 ± 0.81^{d}
NIH standard	0.70–1.20	7.00–20.00	6.00–8.00	3.50–5.00	< 3.00

Means (SEM) with different alphabetical superscripts in the same column are significantly different at $p < 0.05$. Basal: the negative control; WBF1: 85% wheat and 15% of Bambara groundnut flour; WBF2: 76% wheat and 24% of Bambara groundnut flour; WBF3: 53% wheat and 47% of Bambara groundnut flour; WBC1: 85% wheat and 15% of Bambara groundnut concentrate; WBC2: 76% wheat and 24% of Bambara groundnut concentrate; WBC3: 53% wheat and 47% of Bambara groundnut concentrate; Casein: the positive control group; NIH: National Institute of Health.

Table 7. The liver function biomarkers (U/L) in the blood serum of the rats fed with the formulated biscuits.

Samples	AST U/L	ALT U/L	ALP U/L	AST/ALT
Basal	71.30 ± 4.35^{a}	69.82 ± 1.42^{d}	200.25 ± 1.25^{a}	1.02^{a}
WBF1	68.17 ± 1.57^{ab}	86.95 ± 1.52^{a}	119.80 ± 1.25^{b}	0.78^{d}
WBF2	65.35 ± 2.14^{b}	85.21 ± 2.39^{a}	106.56 ± 3.33^{c}	0.76^{d}
WBF3	65.53 ± 3.71^{b}	78.26 ± 1.87^{b}	109.12 ± 1.29^{c}	0.83^{c}
WBC1	55.89 ± 2.86^{c}	72.60 ± 2.83^{c}	59.28 ± 5.71^{e}	0.94^{b}
WBC2	54.46 ± 4.29^{c}	72.17 ± 0.91^{c}	57.76 ± 3.21^{f}	0.75^{d}
WBC3	51.35 ± 7.14^{c}	71.30 ± 1.35^{c}	56.76 ± 0.30^{g}	0.72^{e}
Casein	24.64 ± 2.86^{d}	80.00 ± 1.60^{bc}	99.36 ± 2.14^{d}	0.31^{g}
NIH standard	17.50–30.20	45.70–80.80	56.80–128.00	< 1.00

Means (SEM) with different alphabetical superscripts in the same column are significantly different at $p < 0.05$. Basal: the negative control; WBF1: 85% wheat and 15% of Bambara groundnut flour; WBF2: 76% wheat and 24% of Bambara groundnut flour; WBF3: 53% wheat and 47% of Bambara groundnut flour; WBC1: 85% wheat and 15% of Bambara groundnut concentrate; WBC2: 76% wheat and 24% of Bambara groundnut concentrate; WBC3: 53% wheat and 47% of Bambara groundnut concentrate; Casein: the positive control group; NIH: National Institute of Health.

Table 8. Lipid profile (mg/dL) in the blood serum of the rats fed with the formulated biscuits.

Samples	Cholesterol (mg/dL)	HDL (mg/dL)	Triglyceride (mg/dL)	LDL (mg/dL)
Basal	139.81 ± 0.43^{b}	28.26 ± 9.23^{c}	140.31 ± 12.79^{a}	25.68 ± 2.18^{e}
WBF1	123.88 ± 6.26^{bc}	16.06 ± 6.92^{d}	132.51 ± 7.94^{b}	37.72 ± 3.78^{c}
WBF2	144.78 ± 6.73^{a}	21.01 ± 0.23^{e}	136.25 ± 7.12^{b}	31.10 ± 2.54^{a}
WBF3	125.87 ± 6.78^{bc}	34.32 ± 6.92^{b}	30.44 ± 6.43 ^{c}	24.26 ± 2.51^{e}
WBC1	117.91 ± 4.69^{c}	16.15 ± 3.85^{d}	31.26 ± 7.09^{c}	23.90 ± 4.78^{d}
WBC2	142.79 ± 6.21^{a}	19.15 ± 3.85^{d}	30.39 ± 2.39^{a}	21.76 ± 2.07^{c}
WBC3	145.78 ± 1.99^{a}	22.01 ± 0.91^{e}	138.98 ± 5.74^{a}	19.17 ± 4.09^{b}
Casein	134.36 ± 3.02^{b}	40.38 ± 4.62^{a}	139.87 ± 5.65^{a}	20.80 ± 2.36^{f}
*Ref. Standard	< 200.00	40.00–50.00	< 150.00	< 40.00

Means (SEM) with different alphabetical superscripts in the same column are significantly different at $p < 0.05$. Basal: the negative control; WBF1: 85% wheat and 15% of Bambara groundnut flour; WBF2: 76% wheat and 24% of Bambara groundnut

flour; WBF3: 53% wheat and 47% of Bambara groundnut flour; WBC1: 85% wheat and 15% of Bambara groundnut concentrate; WBC2: 76% wheat and 24% of Bambara groundnut concentrate; WBC3: 53% wheat and 47% of Bambara groundnut concentrate; Casein: The positive control group. * Standard normal range [16].

The total blood protein ranged from 1.93 ± 0.09 g/dL in the basal group to 6.42 ± 0.02 g/dL in the WBC3 group (fed on Bambara groundnut concentrate), which is generally lower than the normal range value of serum protein (6 to 8 g/dL) [16]. The albumin and globulin ranged from 0.66 ± 0.05 g/dL in basal group to 3.92 ± 0.07 g/dL in WBC3 group and 1.88 ± 0.99 g/dL in WBF3 group to 2.65 ± 0.07 g/dL in WBF1 group, respectively, which is lower than the normal range value for serum albumin and globulin (3.50–5.00 g/dL) and (< 3.00 g/dL), respectively. However, for the rats fed diets with incorporated Bambara protein concentrate, the total blood protein values were within required values for experimental animals. The normal rat reference range is between 5.5 g/dL and 7.5 g/dL.

From the experimental biscuit fed to the rats, the creatinine value of the serum ranged from 0.98 ± 0.01 mg/dL in WBC3 group to 2.33 ± 0.05 mg/dL in basal group while that of WBF1 group was 2.15 ± 0.06 mg/dL and WBF2 group was 2.12 ± 0.02 mg/dL. The urea value of the rats fed with the experimental biscuit ranged from 10.57 ± 1.72 mg/dL in WBC3 group to 29.88 ± 1.97 mg/dL in basal diet. In the formulated biscuits group, the creatinine and urea values were within the normal ranges, except for the control sample of 29.88 ± 1.97 mg/dL in basal group. This indicates that the formulated biscuit diet may not have a negative effect on the kidney function except for the basal diet.

ALT, ALP, AST values are different in group WBF2 and WBF3 with values of 106.56 ± 3.33 U/L and 109.12 ± 1.29 U/L, respectively. The AST/ALT ratios of the formulated biscuit fed to the rats ranged from 0.72 in WBC3 group to 0.94 in WBC1 group, which is higher than the positive control (casein) with 0.31 value and lower than the negative control (basal) with 1.02 value. The normal or standard range is (< 1.00). The consumption of this biscuit may be good and may not lead to liver damage from observation. The biscuit from Bambara concentrate substitution had high protein content and also high concentrations of the enzymes that are used to measure liver functionality as shown in Table 5. The AST value ranged from 51.35 ± 7.14 U/L in WBC3 group to 68.17 ± 1.57 U/L in WBF1 group, which is above the positive control of 24.64 ± 2.86 U/L in casein group and the normal AST range value (17.50–30.20 U/L) [16]. The amino ALT value ranged from 71.30 ± 1.35 U/L in WBC3 group to 86.95 ± 1.52 U/L in WBF1 group, which is significantly (p < 0.05) higher than the rat fed with casein. The ALP value ranged from 56.76 ± 0.30 U/L in WBC3 group to 119.80 ± 1.25 U/L in WBF1 group, while the control was 99.36 ± 2.14 U/L in the casein group. The AST/ALT ratio for all the samples are below one which is within the normal range of (< 1.00), an indication that the consumption of the biscuits may not affect the normal functionality of liver.

Lipids profile in the blood serum of the rats fed with the formulated biscuits is shown in Table 6. The results of cholesterol test shows that the values gotten ranged from 117.91 ± 4.69 mg/dL in WBC1 group to 145.78 ± 1.99 mg/dL in WBC3, while the positive control (casein) was 134.36 ± 3.02 mg/dL. There was no significant difference (p > 0.05) among WBF2, WBC2 and WBC3, which had the following values: 144.78 ± 6.73 mg/dL, 142.79 ± 6.21 mg/dL and 145.78 ± 1.99 mg/dL, respectively. All the values for the cholesterol results were within the standard range except HDL values. The HDL values ranged from 16.06 ± 6.92 mg/dL in WBF1 group to 34.32 ± 6.92 mg/dL in WBF3 group, which was lower than that of the control (casein) 40.38 ± 4.62 mg/dL. The LDL values ranged from 19.17 ± 4.09 mg/dL in WBC3 group to 37.72 ± 3.78 mg/dL in WBF1 group, which was relatively higher (p < 0.05) than the control (casein) with value 20.80 ± 2.36 mg/dL. The triglyceride value of the rats fed with the formulated biscuit ranged from 30.39 ± 2.39 mg/dL in WBC2 group to 138.98 ± 5.74 mg/dL in WBC3 group, which had no significant difference with the control (casein) having a value of 139.87 ± 5.65 mg/dL.

The lipids values remained within the normal range for rats, which indicates that the consumption of this biscuit may not pose the risk of cardio-vascular disease (CVD).

Discussion

Results of proximate analysis of biscuit made from the composite flour

The crude protein content reported in this study for WBC3 (36.20%) is higher than the 14.58% reported by Adegbanke et al. [6] and this could be because of the difference in source, origin and processing conditions respectively. This shows that the incorporation of Bambara groundnut flour into wheat flour increased the protein content of the biscuit. Cereal-legumes composite flours have been reported to have increased protein content [4, 17, 18]. Table 3 showed that there is a notable difference at $p < 0.05$ between the samples.

The fat content is very low compared with the values presented by Kiin-Kabari and Giami [13]. It is known that high-level fat content has the potential to alter the storage life of the biscuits due to oxidative activities. Results of statistical analysis showed that there was significant difference between the samples at $p < 0.05$. The result of the crude fiber showed that it ranged from 1.30 ± 0.01% in sample WBF1 to 2.50 ± 0.20% in sample WBC2. From the analysis it could be seen that biscuit made from Bambara protein concentrate had the highest crude fiber because Bambara groundnut has been reported to have a substantial quantity of crude fiber [19]. Results shown from statistical results revealed that there is significant difference between the samples at $p < 0.05$. The ash content ranged from 1.00% in sample WBF1 to 2.80% in sample WBC3. Table 3 showed that there is an increase in ash content as the Bambara groundnut flour increases. The ash content of the formulated biscuit is higher than that of the 100% wheat biscuit. It has been reported by Oyeleke et al. [19] that Bambara groundnut flour had about 3.10 ± 0.10% ash content. From the statistical results, it was revealed that there is a notable difference between the samples at $p < 0.05$. The moisture content ranged from 5.00% in sample WBC3 to 8.69% in sample WF. The moisture content is lower than that of the 100% wheat flour. The moisture content of all the samples was found to be lower than the 10% moisture level standard range for storage stability of flours [20]. In summary, the storage life of products can be elongated with a very low moisture content. Results from statistical analysis show that there is a significant difference between the samples at $p < 0.05$.

The carbohydrate content ranged from 49.50 ± 0.03% in sample WBC3 to 71.03 ± 0.41% in sample WF. Results shown from statistical analysis showed that there is notable difference between the samples at $p < 0.05$. The carbohydrate value of the biscuit samples incorporated with Bambara groundnut flour and protein concentrate was lower than that of the control sample. This could be because the substitution caused a decrease or alteration in the carbohydrate value of the blended wheat-Bambara groundnut sample. Elochukwu and Onyekwelu [21] found that the carbohydrate content decreased in cookies made from a mix of wheat and Bambara groundnut. The energy content of the biscuit ranged from 381.60 ± 1.30 kcal/100 g in sample WF to 399.20 ± 4.20 kcal/100 g in sample WBF1. The formulated biscuit sample energy values are higher than that of the control, which is a 100% wheat flour biscuit. Results shown from statistical analysis showed that there is significant difference between the samples at $p < 0.05$.

Biochemical parameters in the serum of Wistar rats

The total blood protein ranged from 1.93 ± 0.09 g/dL in basal group to 6.42 ± 0.02 g/dL in WBC3 group, which is lower than normal range value of serum protein (6 to 8 g/dL) [16], the albumin and globulin ranges from 0.66 ± 0.05 g/dL in basal group to 3.92 ± 0.07 g/dL in WBC3 group and 1.88 ± 0.99 g/dL in WBF3 group to 2.65 ± 0.07 g/dL in WBF1 group respectively which is lower than the normal range value for serum albumin and globulin (3.50–5.00 g/dL) and (< 3.00 g/dL) respectively [16]. The reduction in these values may be due to low absorption of protein. The low absorption of the protein may be due to the nature of the protein that is found in Bambara groundnut. It has been discovered that the biological accessibility of animal-protein is greater than that of plant-protein. According to Manolis et al. [22], the amount of protein intake and quality will determine plasma protein concentration, especially albumin.

In the formulated biscuits group, the creatinine and urea values are in the normal range [11], except for the control sample of 29.88 ± 1.97 mg/dL in the basal group. This indicates that the formulated biscuit diet may not have a negative effect on kidney function, except for the basal diet. The proper removal or low level

of creatinine and serum urea concentration implies that the kidney is healthy and is functioning well, while high creatinine and serum urea is an indicator of kidney malfunctioning [23, 24].

The normal or standard range for ALT is (45.70–80.80). The consumption of this biscuit may be good and may not lead to liver damage based on observation. The AST value ranged from 51.35 ± 7.14 U/L in the WBC3 group to 68.17 ± 1.57 U/L in the WBF1 group, which is above the positive control of 24.64 ± 2.86 U/L in the casein group and the normal AST range value (17.50–30.20 U/L) [16]. There was a significant (p < 0.05) difference down the column, while there was no significant is rich in sulfur containing amino acids and antioxidants due to the addition of Bambara groundnut concentrate. The AST/ALT ratio for all the samples is below one, which is within the normal range of (< 1.00), an indicator that the consumption of the biscuit may not affect the normal functionality of the liver. From previous scientific studies, it has been observed that high AST or ALT in the blood indicates liver function abnormalities or damage [25–27].

The lipid values remained within the normal range for rats, which indicates that the consumption of this biscuit may not pose the risk of CVD. Normal plasma lipid profiles imply metabolic homeostasis, reducing the likelihood of lipoprotein oxidation and subsequent plaque formation in arterial walls [28]. These may be due to relatively low polyunsaturated fatty acids (PUFAs) content and the presence of sulfur containing amino acids, such as methionine, which can act as a free radical scavenger in Bambara groundnut. A previous study using the Conophor nut model gave similar safe levels for the serum lipid indices [29]. It has been scientifically proven that high level or irregularities of lipids and lipoprotein is associated with increased positive CVDs incidence [30, 31]. The examination of vascular and myocardial tissues would have confirmed the absence of pathological changes, reinforcing the observation of normal lipid values [32].

In conclusion, biscuits were produced by substituting part of the wheat with Bambara groundnut flour and Bambara groundnut protein concentrate. The mixing of Bambara groundnut flour and Bambara protein concentrate significantly improved the protein content, crude fat, total ash, crude fiber, and energy. This research work revealed that Bambara groundnut protein digestibility or bioavailability is low, which might be due to the nature of the protein source (plant source). The urea and creatinine levels were within an acceptable range, which indicates no kidney failure or damage in the animal experiment. The ALT, ALP, AST, which are the enzymes used to measure liver functionality, show that the consumption of this product may not have a negative effect on liver functioning. The cholesterol level, LDL, and HDL levels were low, which indicates from observation that the consumption of this biscuit may not pose the risk of CVD. The samples had high triglyceride values, which are indicative of high energy storage that can be converted for use during fasting. However, out of all the experimental biscuits that were formulated and fed to the rats, sample WBC3 (53% wheat and 47% Bambara groundnut concentrate) was ranked the best in terms of nutritional value, blood protein profile and kidney functioning.

The present study had some limitations and challenges. For example, there was no measurement of food intake for the animal experiment. Also, while carrying out analyses on the experimental animals fed with the biscuits, no histopathological examination of liver/kidney tissues was done. The study did not exceed 21–28 days, which does not give room to check for chronic effects on the animals' organs. Protein digestibility assay (e.g., PDCAAS or in vitro digestibility) was not carried out.

Abbreviations

ALP: alkaline phosphatase

ALT: alanine aminotransferase

AST: aspartate aminotransferase

CVD: cardio-vascular disease

HDL: high-density lipoprotein

LDL: low-density lipoprotein

NaOH: sodium hydroxide

PEM: protein-energy malnutrition

R-T-E: ready-to-eat

Declarations

Author contributions

OEF: Conceptualization, Methodology, Writing—original draft. PJA: Investigation, Resources, Writing—review & editing. ORA: Supervision, Resources, Writing—review & editing. ETA: Formal analysis, Data curation, Writing—review & editing. VNE: Supervision, Project administration, Writing—original draft, Writing—review & editing. All authors read and approved the submitted version.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Ethical approval

The study was approved by the School of Agriculture and Agricultural Technology, Federal University of Technology Akure, Ethical Committee under the ethical number FUTA/SAAT/2024/022, in compliance with the Guide for the Care and Use of Laboratory Animals.

Consent to participate

Not applicable.

Consent to publication

Not applicable.

Availability of data and materials

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

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