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Protein Quality, Beta-Carotene Contents, and Sensory Properties of Maize-Based Snack Enriched with African Yam Bean Seed Flour

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Abstract

Maize-based snacks are deficient in protein and vitamin A, which can be enriched using value-added processes to incorporate appropriate food ingredients such as African yam bean seed flour (AYBSF). African yam bean seed is a nutrient-dense, but underutilised crop. This study was designed to investigate some quality attributes of maize-based snacks enriched with AYBSF, and its application as functional food ingredients. The maize variety containing carotenoids was chosen to produce maize flour (MF), and enriched with AYBSF to produce snacks *(Kokoro*) at various substituted ratios MF/AYBSF (100:0, 80:20, 70:30 and 60:40) using Box Behnken experimental design. Carotenoid contents, trypsin inhibitor activity (TIA), in vivo protein quality and sensory attributes of the samples were determined. The carotenoids contents of the flour blends showed a significant ($p<0.05$) decrease in lutein (7.20-5.11µg/g), zeaxanthin (10.36-6.06 µg/g), β-cryptoxanthin (1.83-1.13 µg/g), α-carotene (0.36-0.21 µg/g) and β-carotene (1.84-0.92 µg/g) as the percentage of AYBSF increased in the flour blends. The TIA was not detected in 100% MF, but TIA increased in the flour blends with the increasing percentage of AYBSF, which reduced significantly during the processing of the flour blends into K*okoro*. The protein availability in *Kokoro depicted* no significant ($p>0.05$) difference from the standard casein diet, which implies that the developed snack compares well with standard casein. This study shows that enriching maize-based snacks with AYBSF is a good prospect which could be useful in advising nutritional programs, especially for school-aged children. *Kokoro* enriched with AYBSF could be preferred to the commonly consumed carbohydrate-based snacks.

Keywords: Maize; African yam bean flour; *Kokoro;* Nutritional quality; Trypsin inhibitor activity

1. Introduction

Carotenoids represent the most ubiquitous group of naturally occurring pigments synthesised only by plants (mainly fruits and vegetables), whose colour tones range from yellow to orange-red such as tomatoes, carrots, and oranges (Melendez-Martinez *et al.,* 2023; Mora *et al.,* 1999). The health benefits of dietary

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carotenoids are well documented ((Basu *et al.*, 2001). Some of the carotenoids such as lycopene have attracted research interest due to their antioxidant abilities and links to reduced chronic disease risks (Giovannucci *et al.*, 1995; Siems *et al.*, 1999; Rodriguez-Amaya, 2021). Lutein and zeaxanthin have important roles in eye health in terms of action against macular degeneration and cataract, which is the leading cause of blindness among the elderly (Brown *et al.*, 1999; Eichler *et al.*, 2002; Wenzel *et al.*, 2003). Protein quality refers to how well a protein source supports the body's needs for growth, maintenance, and repair. It usually gives an indication of how well the body can break down or absorb the protein (Visioli, 2024).

Maize (Zea mays) is the world's third most important cereal after rice and wheat, and the fourth most important cereal in Nigeria (FAO, 2017). It can be used to make snacks, starch, silage e.t.c (Duensing *et al.*, 2003). Snack prepared with 100% maize, lacks the nutritional requirements of the body (Idowu and Aworh, 2017; Awolu *et al.*, 2015). Maize contains a high level of carbohydrates but low protein. Hence, it is very important to enrich maize products with food ingredients that are rich in protein, fibre, and minerals (Udensi, 2001; Idowu and Akinoso, 2016; Galani *et al.*, 2020). There has been research focused on supplementing cereal foods with legumes so as to increase the functionality and nutritional value (Idowu, 2015; Awolu *et al.*, 2016; Ziena, 2022). Other nutrition intervention strategies such as fortification, dietary diversification, and nutrition education have also been implemented (Kaur *et al.*, 2022). Maize (Zea mays) contains a significant amount of lutein, zeaxanthin, and other carotenoids. Processing of maize is used to increase its shelf life, however, a significant loss of nutrients may occur during processing.

African yam bean (*Sphenostylis stenocarpa*) is one of the less utilised legumes that are gradually going into extinction (Klu *et al.*, 2001; NRC, 2007; Idowu and Aworh, 2014; George *et al.*, 2020). This may be due to the long cooking time it requires, and its bitter taste (Shitta *et al.*, 2021) thereby limiting its utilisation. However, it may be processed into different forms to ease the utilisation such as flour for preparing other food products. Small-scale farmers in sub-Subharan Africa are the major cultivators of African yam bean (Adewale *et al.*, 2008; Afolabi *et al.*, 2019). The plant grows wild throughout Tropical Africa, and it is reported to be cultivated in Ivory Coast, Ghana, Nigeria, Togo, Gabon, Congo, Ethiopia, and parts of East Africa (Wokoma and Aziagba, 2001; Aina *et al.*, 2020). When harvested, African yam bean gives an edible tuber and seed (Ajibola and Olapade, 2016). It is well known that African yam bean seeds have high protein content with a relatively high lysine content, which makes them ideal to be combined with cereals (Nwosu, 2013; Anya and Ozung, 2019). Its amino acid profile is similar to that of a whole chicken egg and meets FAO's daily requirements (Ekpo, 2006; Gbenga-Fabusiwa, 2020). Aside its protein content, it also contains appreciable amounts of carbohydrates similar to those in grain cereals (Baiyeri *et al.*, 2018; Adegboyega *et al.*, 2020). Its mineral contents (zinc, magnesium, calcium, iron e.t.c.) are comparable or higher than that of soybean (Adamu *et al.*, 2015). Aside its nutritional content, African yam bean have been found to contain bioactive compounds which are beneficial to health (Ade-Omowaye *et al.*, 2015; Soetan *et al.*, 2018; Edem *et al.*, 2025). Consumption of African yam bean has been found to significantly increase nutrition and food availability (Ukom *et al.*, 2019). African yam bean is very useful as a supplement for starchy foods, such as cassava and maize products (Eneche, 2005; Agbowuro, 2021).

Several researchers have reported increased nutritional content on maize snacks enriched with legumes such as soybean, groundnut, and cowpea (Henshaw and Craig, 1998; Uzo-Peters *et al.*, 2008; Yusufu *et al.*, 2014; Idowu and Aworh, 2017; Ziena, 2022). Idowu (2015), reported improved nutrient composition of maize snacks enriched with African yam bean (a neglected underutilised, but nutrient-dense specie of legume). But there is limited information on the protein quality, trypsin inhibition activity, and carotenoid contents of these products. Although legumes are rich in protein, they contain anti-nutritional factors such as haemagglutinin, tannins, oligosaccharides, and trypsin inhibitor activity (Sam, 2019), whose effect on the products needs to be evaluated. Also, it is essential to evaluate the protein quality of the food which is a measure of the usefulness and availability of food protein to the human body d. Therefore, the present study aimed at evaluating the protein quality, carotenoid content, and sensory properties of maize-snacks enriched with African yam bean seed flour.

2. Materials and Methods

2.1. Materials

Maize varieties, (TZL-Comp4C2 and BR-9928-DMR-SY) were obtained from the International Institute of Tropical Agriculture (IITA), (7.5°N, 4.5°E), Ibadan, Oyo state, Nigeria. A variety of African yam bean seeds (AYBS) identified as Tss-30 (at IITA) used for this study was obtained from local markets. Two commercial maize varieties (dried), BR-9928-DMR-SY and TZL-Comp4C2 were tested for carotenoid contents, the carotenoid-containing maize was chosen and blended with AYBS to produce enriched maize snacks.

2.2. Maize Flour and African yam bean flour preparation

Maize (BR-9928-DMR-SY) seeds were sorted and milled using a hammer mill (model ED-5 Thomas Wiley, England) and sieved using 500 µm mesh size and packaged in high-density polyethylene until when needed. The African yam bean seed flour was produced as reported by Idowu, (2015). African yam bean seeds, AYBS (Tss-30) were thoroughly cleaned to remove dust, stones, debris, and other extraneous materials then, weighed, washed, and dehulled manually by soaking in water at a ratio of 1:5 w/v for about 4 h at room temperature (29±2°C) and dried (60±2°C) in an air draught oven. The dehulled and dried seeds of African yam bean were milled into flour using a hammer mill (model ED-5 Thomas Wiley, England) and sieved using a 500 µm mesh size.

2.3. Maize-African yam bean seed flour blends' preparation

The maize flour was substituted with 0, 20, 30, and 40% African yam bean seed flour (AYBSF) by weights to form different flour blends as earlier reported by ((Idowu (2015). Each blend was properly mixed in a dry-mill blender (Philip blender HR 2611) for about 3 min to obtain a homogenous sample and each sample was packed separately in airtight plastic containers and stored till needed.

2.4. Preparation of enriched *kokoro* from flour blends of maize and African yam bean

Kokoro was produced from various maize-AYBS flour blends. at optimum processing conditions recommended by Idowu and Aworh (2014). These optimum conditions were a frying temperature of 155 °C and a frying time of 11.5 min using 30 percent AYBS flour inclusion in the flour blend. The maize-AYBS flour blends were divided into two equal parts to produce *Kokoro.* The first half was mixed with a measured quantity of salt and onion and the second half was mixed with hot water to make a paste. The two halves were mixed together by continuous stirring using a wooden turning stick for about 3 min to obtain a homogenous dough. The dough was allowed to cool to a temperature of about 40 °C and kneaded and shaped into uniform sizes then, deep fried at 155 °C for 11.5 min, as recommended by Idowu and Aworh (2014). The fried samples were drained of excess oil, left to cool, packaged, and stored at ambient conditions (24±3°C temperature and 61±3% relative humidity).

2.5. Determination of carotenoids contents

Carotenoid analysis was done on each flour blend, using the method of Julie and Sherry (2006). The extraction of carotenoid from dried maize (0.6 g) was done by adding ethanol (6 ml) containing 0.1% BHT and mixing by the vortex. The mixture was subjected to ethanol precipitation for 5 min in the water bath at 85 °C. Potassium hydroxide (500 µl, 80%w/v) was added to the mixture to saponify the interfering oil. Samples were vortexed and placed in a water bath (85°C) for 5 min, vortexed again, and returned to the water bath for an additional 5 min. Upon removal, the samples were immediately placed in an ice bath where 3 ml of cold deionized water was added. Carotenoids were separated 3 times with the addition of 3 ml of hexane, vortexed, and then centrifuged (1200 rpm) for 5 min. The combined hexane fractions were washed with deionised water 4 times, vortexed, and centrifuged for 5 min at 1200 rpm. The hexane fractions were dried down in a concentrator under nitrogen gas. Samples were reconstituted in Methanol/Dichloroethane (1 ml, 50:50 v/v) and 100 µl of the samples were injected into the HPLC. A Waters HPLC system (Waters Corporation, Milford, MA) consisting of a guard-column, C30 YMC Carotenoid column (4.6x250mm, 3µl), Waters 626 binary HPLC pump, 717 auto-sampler, and a 2996 photodiodee array detector was used for carotenoids quantification.

Solvent A: consisted of Methanol/Water (92:8v/v) with 10 mM ammonium.

Solvent B: consisted of 100% Methyl tert-butylether.

Gradient elution was performed at 1 ml/min.

2.6. Determination of Trypsin Inhibitor Activity (TIA)

Trypsin inhibitor activity (TIA) was determined according to a modified method of Shang *et al.* (2016). One (1) gram of already defatted sample (cold extraction) was extracted with 50 ml of 0.01M NaOH. The pH of the suspension was adjusted between 8.4-10.0 using 1M HCl to reduce pH and 1M NaOH to increase the pH to the required level. The sample was left for 3 h, stirring at intervals to maintain the sample in suspension.

The extract (1 ml) was taken into 33 ml of distilled water for dilution, from the diluted extract; 2 ml was transferred into 3 test-tube each. To each of the three test tubes, 2 ml distilled water was added. The fourth test tube was prepared for Trypsin standard by adding 2 ml of distilled water. 2 ml of Trypsin solution (prepared from 4 mg standard trypsin (bovine pancreas, salt-free) in 200 ml of 0.001M HCl) was added to the first 2 test-tubes and not added to the 3rd and 4th test-tubes. The solution in each test tube was vortexed and placed in a water bath at 37^{o}C for 10 min. BAPA (benzoly-DL-arginine-p.nitroanilidehydrochloride) was prepared by dissolving 0.08 g of BAPA in 2 ml of dimethyl sulphoxide and added to the already pre-warmed Tris-Buffer made up of 1.21g of hydroxymethyl amino methane and 0.59 g of $CaCl_2.H_20$ in 180 ml of distilled water, with pH adjusted to 8.2 and the solution finally adjusted to 200 ml with distilled water and pre-warmed in a water bath at 37^{o}C for another 1 h.

BAPA solution (5 ml) was added to all the test tubes, vortexed, and placed in the water bath at 37^{o}C for 10 min. The reaction was terminated exactly 10 min later by the addition of 1 ml of 30% glacial acetic acid solution (30 ml of glacial acetic acid was made up to 100 ml with distilled water to all the test tubes and vortexed. Trypsin solution (2 ml) was added to all the 3rd and 4th test tubes that did not contain Trypsin solution initially. Then the samples were filtered, and the absorbance was read at 410 nm using a spectrophotometer.

Calculations:

$$T.I\ \frac{mg}{g}\ of\ sample = \frac{Abs\ of\ standard - Abs\ of\ sample}{0.019\ x sample\ weight}\ x\ \frac{dilution\ factor}{1x sample\ size\ (ml)} \ldots\ldots\ldots\ldots(1)$$

In 1 g sample: $T.I\ \frac{mg}{g}\ of\ sample = \frac{(Abs\ of\ standard - Abs\ of\ sample) x\ dilution\ factor}{19} \ldots\ldots\ldots(2)$

2.7. Determination of Protein Availability

2.7.1. Biological assessment of the product

A basal diet was prepared according to the method described by Ingale and Shrivastava, (2011). The compositions (g/kg) were fermented corn flour (647.5); glucose (50); sucrose (150); non-nutritive cellulose (microgranular cellulose, 50); vegetable oil (100); premix (20); oyster shell (10); bone meal (20) and sodium chloride (2.5). Experimental and control diets were prepared by incorporating fried AYB-maize rings and casein (for control) respectively into the basal diet to achieve an iso-nitrogenous diet at 10% protein level as presented in Table 1 using equations (3) and (4);

$$a\ x\ \frac{X}{100} = 10\ x\ \frac{Y}{100} \qquad \ldots\ldots\ldots\ldots(3)$$

$$X = \frac{10Y}{a} \qquad \ldots\ldots\ldots\ldots(4)$$

(Where X is weight of test sample required for the feed mixture, Y is final weight of feed mixture, a is protein content of test sample, 10 is iso nitrogenous protein required)

The Feed Efficiency Ratio (FER), Protein Efficiency Ratio (PER), the Net Protein Retention ratio (NPR) Protein Retention Efficiency (PRE) and Protein Conversion Efficiency (FCE) were calculated using the formulae given below (Eqns 5-9) as reported by Osundahunsi and Aworh (2003).

$$FER = \frac{Wgained}{food\ intake\ (g)} \qquad (5)$$

$$PER = \frac{Wgained}{P} \qquad (6)$$

$$NPR = \frac{Wgained - Average\ Wloss}{P} \qquad (7)$$

$$PRE = NPR x 16 \qquad (8)$$

$$FCE = \frac{daily\ protein\ intake(g/day)}{daily\ Weight\ gained\ (g/day)} \qquad (9)$$

(Where W gained is weight gained by the rats after feeding, W loss is weight loss by the rats fed basal diet, P is protein consumed by animals). P

2.7.2. Nutritional Evaluation of the Products

Twelve Wistar rats (male) weighing between 90 g and 110 g were obtained from the animal breeding centre, Department of Veterinary Physiology and Pharmacology, Faculty of Veterinary Medicine, University of Ibadan, Ibadan. They were randomly distributed into three groups each consisting of 4 replicates placed in metabolic cages and fed a stabilising diet consisting of 4% casein for a period of 5 days. After the 5-day period, the animals were reweighed and regrouped for control, basal and experimental diets. Water and food were given *ad libitum*. The diets were fed to the animals for a period of 28 days. This period is nutritionally accepted to be long enough to observe biological and chemical changes in animal tissues (Osundahunsi and Aworh, 2003; Ikya *et al.,* 2013). Weighed diet was given and the unconsumed diet was collected and weighed daily, while the live weight of the animals was determined and recorded twice a week throughout the experimental period. At the end of test period, the rats were reweighed. The experimental animals were starved for 12 hours and were sacrificed thereafter.

2.8. Sensory evaluation

The sensory evaluation was carried out using semi-trained panelists, who were selected from members of staff and graduates of the International Institute of Tropical Agriculture (IITA), Ibadan, Oyo state, Nigeria. The screening of the panelists was based on their interest and ability to differentiate food sensory properties as described by Iwe, (2002). Each panelist was provided with a glass of clean water to rinse their mouths between the four evaluation sessions of 3 min interval. A total of four samples including the 100 % maize flour *Kokoro* samples were presented in two-digit coded plates and were evaluated for appearance, texture/crispiness, aroma, taste and overall acceptability, using a nine-point hedonic scale in which the least score was indicated with 1 (dislike extremely) and the highest score with 9 (like extremely) for all the attributes (Iwe, 2002, Sahu, 2020).

2.9. Statistical Analysis

All data generated from the study were subjected to Analysis of Variance (ANOVA) using SPSS software (version 21.0). Means were separated using LSD and all data were statistically analyzed at $p<0.05$.

3. Results and Discussion

3.1 Carotenoid contents of maize samples

The carotenoids present in the maize flour (MF) and African yam bean seed flour (AYBSF) blends are presented in Table 2. Carotenoids were not detected in the TZL-Comp4C2 maize variety and 100% AYBF, but appreciable levels of carotenoids were found in the BR-9928-DMR-SY maize variety. Carotenoid contents of the flour blend produced from various ratios of MF-AYBSF (80:20, 70:30, and 60:40) had 6.29, 5.28, and 5.11 (lutein), 8.06, 6.55, and 6.06 (zeaxanthin), 1.49, 1.25 and 1.13 (α-cryptoxanthin), 0.13, 0.21 and 0.21 (β-cryptoxanthin), 0.13, 0.04 and 0.09 (α-carotene) and 0.88, 0.74 and 0.68 (β-carotene) respectively. The carotenoid contents were found to decrease with increasing quantity of AYBSF in the flour blends. The analysis of the two common varieties of maize, BR-9928-DMR-SY and TZL-Comp4C2 tested for carotenoids contents showed that BR-9928-DMR-SY represents a high pigmented (yellow) maize variety and TZL-

Comp4C2 represents a very low-pigmented (white) maize variety. Total carotenoids contents of 21.03 μg/g were obtained in the maize samples of BR-9928-DMR-SY, this result is consistent with the report of Tawanda et al. (2011) that yellow-pigmented maize contains lutein content in the range of 0.88 to 41.85 μg/g, 0.74 to 30.68 μg/g for zeaxanthin content, and 0.05 to 16.79 μg/g for β-carotene. Munkhuwa et al. (2023), also reported similar values for betacarotene contents of two Provitamin-A maize varieties. The major carotenoids present were lutein and zeaxanthin. Detectable levels of α-, β-cryptoxanthin, α, and β-carotene were also found. The most abundant carotenoid in BR-9928-DMR-SY maize was zeaxanthin, comprising 49.26% of total carotenoids followed by lutein. This may be because carotenoids were not detected in the 100% AYBSF (Table 2). However, the need to increase the protein content of maize flour necessitates its enrichment with nutrient-dense crops like AYBSF. Combined with the optimisation of the snack production to attain the processing conditions with the best quality products.

Table 2: Carotenoids contents (μg/g) of maize flour (MF) and African yam bean seed flour (AYBSF) blend

Sample ID MF: AYBF (%)	lutein	zeaxanthin	α-cryptoxanthin	β-cryptoxanthin	α-carotene	β-carotene	Total carotene
80:20	6.29^{b}	8.06^{b}	1.49^{b}	0.31^{b}	0.13^{b}	0.88^{b}	17.16^{b}
70:30	5.28^{c}	6.55^{c}	1.25^{c}	0.21^{c}	0.04^{c}	0.74^{c}	14.07^{c}
60:40	5.11^{c}	6.06^{d}	1.13^{d}	0.21^{c}	0.09^{c}	0.68^{c}	13.28^{c}
100% (YMF)	7.2^{a}	10.36^{a}	1.83^{a}	0.36^{a}	0.16^{a}	1.12^{a}	21.03^{a}
100% (WMF)	ND	ND	ND	ND	ND	ND	ND

Values with different subscript on the same column are significantly different $p<0.05$

Where,

AYBSF = African yam bean seed flour

WMF= White maize flour

YMF= Yellow maize flour

ND= Not detected

3.2 Trypsin Inhibition Activity of Samples

The Trypsin inhibitor activity (TIA) of the samples is presented in Table 3. TIA contents in 100%, 20%, 30% and 40% AYBSF are 11.09%, 2.95%, 4.25% and 6.73% respectively, while the TIA content for kokoro sample increases (2.42, 3.60 and 4.08) as the percentage of African yam bean seed flour (20%, 30% and 40%) increases. Trypsin inhibitor is an anti-nutritional factor commonly found in tropical legumes. While TIA was not detected in 100% maize flour, about 11.09% was obtained in 100% AYBSF (Table 3). This is higher than the value (22.09TUI/g) reported for raw whole seeds of African yam bean by Nwosu (2013), but comparable to the value (11.64TUI/g) reported by the same author for the 96 hours malted sample of the African yam bean. The TIA increased in the flour blends as the percentage of the AYBF increased but a significant reduction was observed during the processing of the flour blends into *Kokoro* products. Heat treatment is reported to reduce trypsin inhibitors in foods. Luo et al. (2025), reported appreciable reduction in TIA values during thermal processing of soybean. Bio-processing treatment have also been reported to impact significant reduction to anti-nutritional properties of black wheat, barnyard millet, and black soybean (Goel *et al.*, 2025). Olapade and Aworh (2012) reported a significant reduction of TIA during extrusion cooking of *acha*/cowpea mixtures. This implies that heat processing destroys a significant proportion of TIA and thus poses no serious problem to the utilisation of the African yam bean seeds. The results obtained in this study corroborate the report of Onuoha et al (2017) on the evaluation of nutrients and anti-nutritional factors of different species of African yam bean. Other similar results were captured in the review article of George et al. (2020).

Table 3: Trypsin Inhibitor Activity (%) (TIA) of flour blends and *kokoro* samples.

Flour Blend Sample MF: AYBF (100%)	TIA (%)
100: 0	ND
0: 100	11.09[a]
80: 20	2.95[f]
70: 30	4.25[c]
60: 40	6.73[b]
***Kokoro* Sample**	
80: 20	2.42[g]
70: 30	3.60[e]
60: 40	4.08[d]

Values with the different subscript along column are significantly different at $p<0.05$

A (0% AYBSF); B (20% AYBSF); C (30% AYBSF); D (40% AYBSF)

3.3 Biological Evaluation of Snack from Flour Blends of Maize and African yam bean seed

The result of the biological evaluation of the protein quality of *Kokoro* produced from blends of maize and AYB, basal and casein diet (control) are presented in Table 4. Feed efficiency ratios were 0.32, 0.26 and 0.08 for casein, *kokoro* and basal diets respectively. Similar results were reported for maize-based complementary foods enriched legumes by earlier authors (Osundahunsi and Aworh, 2003; Omosebi and Osundahunsi, 2021; Shadrack *et al.,* 2025). There were significant differences (p<0.05) in feed efficiency ratio (FER), protein efficiency ratio (PER), net protein ratio (NPR), protein retention efficiency (PRE), and feed conversion ratio (FCR). The *Kokoro* had lower values than the casein control diet but higher values than the basal diet. The result is similar to that obtained by Olapade and Aworh (2012) for complementary snacks from cowpea and *acha* blends. Results of the weight of organs of animals fed with the different diets are presented in Table 5. There were no significant differences (p≥0.05) among the weight of the liver, kidney, spleen, and pancreas of rats fed on casein and the "formulated" *Kokoro*. However, the weight of the organs of the animals fed with basal diet exhibited a significant difference (p<0.05) when compared with those of animals fed with the formulated *Kokoro*. The animals fed with the formulated *Kokoro* exhibited increased organ weights than those fed on a basal diet. Kurniawan et al. (2023) also reported increased organ weight for chicken fed with different protein levels. This implies that the increased organ weight may be attributed to higher protein content of the formulated sample. In this study, Casein (control) diet was compared with the experimental diet. The result obtained showed no significant difference between the experimental diet and the casein control diet (Table 4).

The result showed that as the percentage of African yam bean seed flour (0%, 20%, 30% and 40%) in the diet increased, the Feed Efficiency Ratio (0.25 to 0.30), Efficiency ratio (2.00 to 2.5), Protein Efficiency ratio (2.28 to 2.77), Net protein retention (28.85 to 51.36) and Protein weight feed gain/day conversion (0.98 to 7.50) increased also. This is similar to the result obtained by Abeey and Berezi (1998) on the "influence of processing on the protein digestibility of African yam bean seed". This may be attributed to increased protein level as reported by Kurniawan et al. (2023).

3.4 Sensory Evaluation of the Maize-AYB Snacks

The sensory evaluation of the "developed" maize-AYB snacks revealed in Table 6. Significant differences were observed in the colour, taste, aroma, and overall acceptability of the samples as the addition of AYBSF increased, while no significant difference was observed for crispiness. The values obtained for colour did not show any significant difference, however, the *kokoro* enriched with the AYBSF gave a brighter and a more acceptable colour than that of the 100% *kokoro*. This increased as the percentage of AYBSF inclusion in the flour blend increased. This may be attributed to the variety of African yam bean seed used in the study.

Table 4: Biological evaluation of experimental diet compared

Sample ID	Feed Efficiency ratio (FER)	Efficiency ratio(PER)	Protein PER** Efficiency	Net Protein Retention (g)	Protein Conversion efficiency
A	0.25±0.01^{b}	2.00±0.02^{b}	2.28^{b}	28.85±0.02^{b}	0.98±3.72^{b}
B	0.26±0.02^{b}	2.03±0.02^{b}	2.30^{b}	35.38±0.01^{b}	1.22±4.10^{b}
C	0.29±0.02^{a}	2.31±0.01^{a}	2.61^{a}	44.00±0.03^{a}	4.71±3.98^{a}
D	0.30±0.01^{a}	2.32±0.02^{a}	2.66^{a}	48.72±0.02^{a}	5.93±7.56^{a}
Casein	0.32±0.03^{a}	2.50±0.02^{a}	2.77^{a}	51.36±0.01^{a}	7.50±3.64^{a}
Basal	0.08±0.02^{c}	-0.60±0.01^{c}	0.01^{c}	-	0.42±16.54^{c}

Values with the same letter along the same column are not significantly different at $p \geq 0.05$

A (0% AYBSF); B (20% AYBSF); C (30% AYBSF); D (40% AYBSF)

**Based on value 2.5 as standard casein

Table 5: Organ weight of animals fed with the experimental diet

Sample ID	Liver (g)	Kidney (g)	Pancrease (g)	Spleen (g)	Stomach (g)	Intestine (g)	Heart (g)
A	2.82±0.02^{b}	0.87±0.03^{a}	0.10±0^{b}	0.24±0.01^{b}	0.52±0.02^{b}	5.27±0.06^{b}	0.26±0.01^{b}
B	4.07±0.03^{a}	0.93±0.05^{a}	0.27±0.01^{a}	0.40±0.01^{a}	0.92±0.02^{a}	6.33±0.10^{b}	0.38±0.01^{a}
C	4.56±0.04^{a}	0.98±0.04^{a}	0.29±0.02^{a}	0.42±0.01^{a}	1.00±0.01^{a}	8.44±0.08^{a}	0.39±0.01^{a}
D	4.70±0.04^{b}	0.98±0.03^{a}	0.30±0.01^{a}	0.46±0.01^{a}	1.28±0.01^{a}	8.51±0.05^{a}	0.43±0.01^{a}
Casein	4.80±0.04^{a}	1.15±0.03^{a}	0.31±0.01^{a}	0.52±0.02^{a}	1.22±0.01^{a}	7.65±0.05^{a}	0.38±0.01^{a}
Basal	2.29±0.01^{b}	0.54±0.04^{ab}	0.10±0.01^{b}	0.16±0^{b}	0.47±0.01^{b}	3.68±0.06^{c}	0.28±0.02^{b}

Values with the same letter along the same column are not significantly different at $p \geq 0.05$

A (0% AYBSF); B (20% AYBSF); C (30% AYBSF); D (40% AYBSF)

There was no significant difference in the values obtained for the taste and aroma of the samples, as the same quantity of ingredients were used in the preparation of the samples. However, a higher value recorded for the samples containing AYBSF could be attributed to the fact that AYBSF imparts a characteristic taste/flavour similar to that of bean cake, *akara*, which the sensory panelists used for this study must have been familiar with and therefore might have positively affected their rating of samples with AYBSF. The values obtained for the general acceptability, though not significantly different, seems to increase with higher inclusion of the AYBSF. This might mean that the panelists must have perceived the developed product as a blend between the commercial *kokoro* and the widely consumed bean cake known as *akara*.

Table 6: Sensory evaluation of the experimental diet

Samples	Colour	Taste	Crispiness	Aroma	Overall acceptability
A	6.9^{a}	7.0^{a}	6.8^{a}	6.7^{b}	6.9^{a}
B	7.2^{a}	7.5^{a}	7.3^{a}	7.5^{a}	6.9^{a}
C	7.5^{a}	7.8^{a}	7.9^{a}	7.8^{a}	7.6^{a}
D	8.0^{a}	8.1^{a}	6.9^{a}	8.2^{a}	7.8^{a}

Values with the same letter along the same column are not significantly different at $p \geq 0.05$

A (0% AYBSF); B (20% AYBSF); C (30% AYBSF); D (40% AYBSF)

4. Conclusions

The study has shown that maize snack enriched with African yam bean seed flour have increased nutritional content, particularly in terms of protein quality. The protein quality of the enriched maize snack is comparable with that of the casein, the control diet; hence it has the potential to be commercialised and used to alleviate the protein-energy malnutrition among school-aged children in Soth-Western Nigeria.

Conflict of interest: The authors declare that they do not have any conflict of interest.

Ethical statement: The study was approved by the ethical review board of the College of Medicine, University of Ibadan, approval number-CMUL/ACUREC/09/14/926 30/09/2014

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