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BIOLOGICAL EVALUATION OF PROTEIN QUALITY OF PROCESSED AFRICAN WALNUT SEED KERNEL USED AS TRADITIONAL SNACK IN PARTS OF NIGERIA

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ABSTRACT

The protein bioassay of processed African walnut seed kernel used as traditional snack in parts of Nigeria was conducted. Diets formulated with raw and processed seed kernels as the sole protein (12%) sources, together with reference diet were presented to rats *ad labitum* for a 28-day feeding period. The growth performance characteristics: Body weight change, Feed intake, Nitrogen intake, Protein intake, Feed utilization, Feed conversion ratio, Protein efficiency ratio (PER), were significantly ($p<0.05$) higher in rats fed reference diet than those fed sample-based diets. On the other hand, the protein quality indices: Faecal nitrogen, Carcass weight, Faecal dry weight, Net protein utilization, Apparent digestibility, True digestibility, Biological value (BV) of rats fed sample-based diets while not affected considerably by the processing method were statistically ($p>0.05$) similar to those fed reference diet (Nutrend®). The PER and BV of rats adapted on processed samples were above the values 2.0 and 70 respectively required for good quality protein. It could therefore be concluded that the protein of the processed African walnut (*Plukenetia conophora*) kernel used as traditional snack in parts of Nigeria is adequately utilized, capable of supporting life and of acceptable quality.

KEYWORDS: Biological value; bioassay; growth performance; diet; snack.

INTRODUCTION

According to Pellett and Young,[1] the growing imbalance between world food supplies and population growth makes chronic food shortages commonplace. But the match between dietary supply and human protein needs is vital to support the health and well-being of human populations.[2] Pellett and Young[1] also stated that the ability of a food to satisfy human protein needs is a function of both the amount of protein in the food and its nutritional quality. These emphasize the need to utilize available food resources as efficiently as possible and thus the importance of protein quality and its evaluation. The value of a protein is determined by its content of essential amino acids thus, protein quality could be estimated through chemical score in which the amino acid content of the protein source is compared with that of a reference protein.[3] The usefulness of protein in animal nutrition is partly quantitative and partly qualitative. This was succinctly expressed by Hackler[4] when he stated that biological evaluation (bioassay) of protein requires both estimate of protein content as well as an evaluation of the amino acids present and their bioavailability. Bioavailability refers to the proportion of the total amount of dietary amino acids that is absorbed in a form that can be utilized for body protein synthesis and other pathways which constitute the metabolic demand.[2] Bioassay has been acknowledged the best tool for judging the quality of protein, according to Omer *et al*.[5] since numerous factors decide the ultimate *in-vivo* quality of the protein. It involves measuring and comparing the magnitude of the response of the test with that of standard in a suitable biological system under standard set of conditions.

The seed kernels of African walnut (AW) are widely eaten raw, roasted or cooked as masticatories/traditional snacks in West and Central Africa.[6] African walnut (*Plukenetia conophora*) is a climbing *Euphorbiaceae* over 30m long, indigenous to Africa, especially West Africa. It is known in Nigeria as “Ukpa” (Igbo), “Asala” or “Awusa” (Yoruba), “Okhue or Okwe” (Edo). The seed kernel is contained in a thin brown shell resembling the template walnut.[7] As reported by Onyeike *et al*[8] the plant food is rich in protein (26.2±0.61 - 27.7±0.68 g/100g sample) and according to Anyalogbu *et al*.[3] has high proportion of essential amino acids like leucine

(4.50-7.80 g/100 g protein), lysine (3.65-7.09 g/100 g protein) and arginine (3.22-6.12 g/100 g protein) with glutamic acid (7.88-18.5 g/100 g protein) and aspartic acid (4.86-9.16 g/100 g protein) as the most abundant non-essential amino acids. However, the work of Anyalogbu and Ezejiofor[6] indicated the presence of some antinutrients or anti -nutritional factors (such as saponins, alkaloids, cyanogenic glycosides and oxalate) in the plant food. Although the detected antinutrients, according to the researchers were within tolerable limits and were further reduced by the processing method, the possibility of negative effect of the residual quantity on the nutritional value (protein quality) cannot be ruled out. In addition, Boye *et al*.[9] opined that processing conditions and preparation methods are among the factors that affect protein quality. This, Audrey *et al*.[10] reported could be beneficial or detrimental. This work is therefore intended to use *In vivo* Bioassay Technique to estimate the quality of the sample protein while adopting Graded Response Assay in evaluating the effect of processing on the quality parameters.

MATERIALS AND METHODS

Sample Processing

Wholesome seeds collected from fresh African walnut fruit capsules bought from a farmer at Ojoto, Idemili South Local Government Area of Anambra State, Nigeria were used. The seeds were washed severally with distilled water and divided into four portions. The first portion (labelled AW_{raw}) was used raw. This and the other portions (2nd, 3rd and 4th) subjected to graded cooking time and lebelled AW_{45}, AW_{90}, and AW_{135} respectively were further processed into powder as described by Anyalogbu *et al*.[7]

Biological Evaluation

The samples i.e. AW_{raw}, AW_{45}, AW_{90} and AW_{135} were used in formulating diets (Diet 1-4) and then subjected to biological evaluation through rat feeding studies.

Formulation of the experimental diets

Diets were compounded using the ingredients as shown in Table 1.

Table 1: Composition of formulated diets (g/Kg).

	Diets					
	1	**2**	**3**	**4**	**5**	**6**
Components(g)	AW_{raw}	AW_{45}	AW_{90}	AW_{135}	NCD	RD
Sample	382	382	372	361	-	-
Sucrose	113	113	113	113	113	-
Red palm oil	5	5	5	5	5	-
Vitamin and mineral mix	25	25	25	25	25	-
Non-nutritive cellulose	25	25	25	25	25	-
[a]Nutrend(R)	-	-	-	-	-	1000
Corn flour	450	450	460	471	837	-
Total (Kg)	**1.0**	**1.0**	**1.0**	**1.0**	**1.0**	**1.0**
NCD = Negative control diet. [a]RD = Reference diet						

The samples were included in the diets (Diets 1-4) as the sole protein sources. The protein contents of African walnut ranged from 26.2±0.61 to 27.7±0.68 g/100.[8] The samples were added at the expense of corn starch to give approximately 120g protein per kilogram diets.

Negative control (NC) diet, which was the basal or protein-free diet, contained none of the samples. Other ingredients used in formulating the diets included corn starch (as the main source of carbohydrate), red palm oil (to provide dietary fat), non-nutritive cellulose (sieved fine wood shavings added to provide rouphage/dietary fibre), granulated cane sugar/sucrose which was added to enhance palatability of the diets and vitamin and mineral premix (as source of micronutrients). The sucrose was added at the expense of corn starch. Nutrend® (a commercial infant formula) was used as such as a positive control/reference diet (RD).

Proximate composition and energy values of the compounded diets are given in Table 2.

Table 2: Proximate composition and energy values of experimental diets.

Diet	Nutrient composition (g/100g) / energy value(Kcal/100g sample)				
	Protein	**Fat**	**Carbohydrate**	**Energy value (Kcal/100g sample)**	**Protein-Energy ratio**
AW_{raw}	12.26	19.52	60.16	465.36	0.03
AW_{45}	12.26	20.67	59.37	472.55	0.03
AW_{90}	12.31	19.73	60.69	469.57	0.03
AW_{135}	12.36	19.06	61.54	467.4	0.03
NCD	4.19	0.50	94.0	401.26	0.01
RD	15.0	9.0	64.2	397.80	0.04
NCD = Negative control diet, RD = Reference diet					

Experimental design
Based on the recorded initial weights, the rats were allotted to six groups of 6 rats per group such that the initial mean weights of the groups were equalized as nearly as possible and housed individually in all-wire cages with raised platform. After acclimatization, rats adapted to Diets 1-4 received AW_{raw}, AW_{45}, AW_{90} and AW_{135} respectively. Negative control rats received Diet 5 (protein-free) while positive control rats were fed Diet 6 (Nutrend®). The six rats in each diet group were labeled 'A' to 'F'. For example, Rat 1A, 1B, 1C, 1D, 1E and 1F were fed Diet 1. Three hundred grammes of each diet was measured out and labeled 'A' to 'F' such that for Diet 1, there was Diet 1A, 1B, 1C, 1D, 1E and 1F. Feed and water were given to the rats *ad labitum* for the 28-day feeding period. Spilt feed (SF) from each rat was collected and weighed to calculate the quantity of feed consumed by each rat. Faecal droppings of each of the rats were also collected daily and bulked-up in an open container and dried in an oven. At the end of the 28-day feeding period, 6hr fast was imposed on the animals and then their weights taken to obtain the final body weights of the rats.[11]

NUTRITIONAL INDICES

Determination of feacal and carcass N_2: The bulked faecal droppings of each rat was further dried in an oven overnight at 80°C, weighed and ground, and then analyzed for nitrogen content using the Kjeldahl method[12] to obtain the faecal nitrogen. Each carcass was dried to a constant weight in an air- circulatory oven set at 80°C, weighed, ground in a mill and then analyzed for nitrogen content[12] to obtain carcass nitrogen.

Calculation of Nutritional Parameters
Feed intake
Feed intake (FI) describes the amount of feed voluntarily consumed by an animal.[13]

The spilt feed, (collected over the 28-day feeding period) and the remnant (at the end of the 28-day study) of the feed measured out, for each rat was dried and weighed. The sum of the weights was subtracted from the initial weight of the feed measured out for the rat. The difference represented the feed intake (FI) of the individual rat for the 28-day feeding period.[14]

Protein intake
Protein intake per rat for the 28-day study period was calculated by multiplying the feed intake of the rat by the percentage protein content of the diet consumed.[15]

Body weight change
Body weight change (BWC) was calculated as the difference between the weight (g) of the rat at the end of the 28-day feeding period and weight at the commencement of the feeding with the experimental diet.[15]

Feed utilization (FU)
This was calculated for each rat as mg body weight gain per gramme feed consumed in the 28-day feeding period.[15]

i.e. $$FU = \frac{\text{Weight gain(mg)}}{\text{Quantity of dried feed consumed (g)}}$$

Feed conversion (Efficiency) ratio (FCR or FER)
This was calculated as the ratio of gramme body weight gain per gramme feed consumed.[15,16]

i.e. $$\text{Feed conversion ratio} = \frac{\text{bodyweight gain(g)}}{\text{Quantity of dried feed consumed (g)}}$$

Protein efficiency ratio (PER)
This was calculated by the method of Acton and Ruud[17] as described by Wan-Rosli, *et al.*[17] as gramme body weight change per gramme of protein consumed.
i.e.
$$PER = \frac{\text{Weight(g) (gain/Loss) at the end of the feeding period}}{\text{Weight(g) of protein consumed}}$$

Net protein utilization (NPU)
Net protein utilization is the measure of fraction of the nitrogen intake that is retained. It was calculated as described by Bender and Miller[18]:

$$NPU = \frac{B - (Bk - Ik)\ X\ 100}{I}$$

Where B and Bk are the total body (carcass) nitrogen of animals on the test and non-protein (Basal) diets respectively while I and Ik are the intake of nitrogen in the two rat groups.

Apparent (Protein) digestibility
An apparent digestibility estimate what is left when nutrients contained in the faeces is subtracted from nutrients contained in the dietary intake.

Apparent digestibility (AD) of protein (N_2 x 6.25) was calculated by measurement of N_2 –intake and amount of N_2 in the faecal droppings using the formula of Bender and Doell.[19]

i.e. $$AD\ (\%) = \frac{\text{N intake(g)} - \text{faecal N(g)}}{\text{N intake(g)}}\ x\ \frac{100}{1}$$

True (Protein) digestibility
A true digestibility estimate what is left when nutrients contained in the faeces (that actually originated from the dietary intake) is subtracted from nutrients contained in the dietary intake. Thus it involves correcting for the endogenous and microbial amount of the nutrient actually lost in the faeces.

True (Protein) digestibility was calculated using the method of Miller and Bender.[20]

i.e $$TD\ (\%) = \frac{I - (F - Fk)\ x\ 100}{I}$$

Where F and Fk are the faecal nitrogen values of animals on the protein and non-protein diets respectively, and I is the nitrogen intake of animals on the protein diets.

Net protein utilization (NPU) / Biological value (BV)
The biological value examines nitrogen balance. The test relates to the body's ability to digest, absorb and excrete given proteins, which are the body's source of nitrogen.

According to Miller and Bender[20] and Platt *et al.*,[21] net protein utilization (NPU) of a protein is the product of its Biological value (BV) and True digestibility (TD) divided by 100.

i.e. $NPU = \frac{BV \times TD}{100}$

Hence, $BV = \frac{NPU}{TD} \times 100$

Statistical Analysis: Test for statistical significance was done using one-way analysis of variance (ANOVA) and treatment means compared by the Duncan's[22] multiple range tests utilizing statistical package for social sciences (SPSS) version-20. All values were expressed as Mean ± SD. Differences in the means were considered significant at $p < 0.05$ in all cases.

RESULTS AND DISCUSSION

The growth performance characteristics of rats fed diets formulated with raw and processed African walnut seed kernel flour as the sole protein (12%) source are presented in Table 3. The BWC, FI, NI, PI, FCR and PER of rats adapted to the raw sample-based diet were statistically ($p<0.05$) lower than those of the cooked samples. These parameters including FU were in-turn significantly ($p<0.05$) higher in rats fed reference diet than those fed sample-based diets.

Table 3: Growth performance characteristics of rats fed diets based on raw and cooked African walnut seed kernel powder.

Parameter	Diet					
	AW_{raw}	AW_{45}	AW_{90}	AW_{135}	NCD	RD
Body weight change (g) (BWC)	45.54 ± 2.52^{c}	72.29 ± 16.69^{d}	67.04 ± 10.10^{d}	65.82 ± 6.14^{d}	8.17 ± 1.62^{a}	116.86 ± 24.45^{b}
Feed intake (g) (FI)	199.35 ± 8.0^{c}	242.17 ± 0.29^{d}	236.67 ± 2.08^{d}	242.67 ± 4.01^{d}	176.17 ± 5.11^{a}	291.17 ± 18.25^{b}
Nitrogen intake (g) (NI)	3.91 ± 0.16^{c}	4.75 ± 0.01^{d}	4.66 ± 0.04^{d}	4.80 ± 0.08^{d}	1.18 ± 0.03^{a}	6.95 ± 0.45^{b}
Protein intake (g) (PI)	24.44 ± 0.98^{c}	29.69 ± 0.04^{d}	29.26 ± 0.28^{d}	30.0 ± 0.50^{d}	7.39 ± 0.21^{a}	43.50 ± 2.94^{b}
Feed utilization (mg/g) (FU)	228.43 ± 19.82^{c}	298.26 ± 69.11^{d}	283.16 ± 42.82^{cd}	271.25 ± 28.33^{cd}	46.43 ± 9.99^{a}	401.14 ± 57.18^{b}
Feed conversion ratio (FCR)	0.22 ± 0.02^{d}	0.30 ± 0.15^{bc}	0.28 ± 0.05^{cd}	0.27 ± 0.03^{cd}	0.05 ± 0.01^{a}	0.40 ± 0.05^{b}
Protein efficiency ratio (PER)	1.86 ± 0.19^{d}	2.43 ± 0.57^{c}	2.29 ± 0.34^{cd}	2.20 ± 0.23^{cd}	1.11 ± 0.24^{a}	2.67 ± 0.37^{b}

Values are means and standard deviation (n = 6). Means in the same row with different superscript letter are significantly different from one another at 5% level ($p<0.05$). NCD = Negative control diet, RD = Reference diet (Nutrend®).

The protein quality indices on the other hand are represented in Table 4. The FN, CW, FDW and BV of rats fed sample-based diets were statistically ($p>0.05$) similar to those fed reference diet.

From Tables 3 and 4, relative to the raw sample (AW_{raw}) cooking increased the BWC, FI, NI, PI, CN, FU, FCR, PER, AD and TD (with the increase in BWC, FI, PI, FCR and PER being significant, $p<0.05$); and decreased the FN, NPU and BV of rats fed diets formulated with African walnut seed kernel flour.

Rats fed the reference diet (RD) showed better performance over the others fed diets formulated with the samples with the BWC, CN, FU, FCR, PER and AD at 95% confidence being statistically higher; whereas rats fed negative control diet (NCD) exhibited poor performance.

The better performance of rats fed the reference diet over those fed the sample diets may be attributed to the fact that the reference diet had higher protein - energy ratio than the compounded diets (Table 2). The decreased feed intake observed in rats fed the sample diets, especially AW_{raw}, may have led to inadequate supply of energy and protein required for normal growth.[1,23]

The presence of antinutritional factors (ANFs) in the raw samples and their residual concentrations in cooked samples, as reported by Anyalogbu and Ezejiofor,[6] may have contributed to the observed low performance of rats on test samples' diets relative to reference diet.[2] Based on the tolerable limits of the antinutrients, the plant foods at all levels of processing may be considered safe for human,[6] nonetheless some ANFs are known to reduce bioavailability of minerals and other nutrients and cause growth inhibition[24,25] either by affecting feed intake and palatability, digestibility or post absorptive utilization.[26,27]

Table 4: Protein quality indices of raw and cooked African walnut seed kernel based diets fed to rats.

	Diets					
Quality index	**AW_{raw}**	**AW_{45}**	**AW_{90}**	**AW_{135}**	**NCD**	**RD**
Carcass nitrogen (g) (CN)	8.07±0.47[c]	8.71±0.87[c]	8.59±0.76[c]	8.40±0.64[c]	6.01±0.43[a]	10.42±0.83[b]
Faecal nitrogen (g) (FN)	0.58±0.12[b]	0.52±0.05[ab]	0.49±0.04[ab]	0.50±0.60[b]	0.36±0.02[a]	0.51±0.25[b]
Carcass weight (g) (CW)	122.15±14.95[ab]	124.03±17.75[ab]	122.01±16.91[ab]	117.56±9.30[ab]	85.55±4.40[a]	142.68±39.39[b]
Faecal dry weight (g) (FDW)	21.50±2.50[a]	21.17±1.26[a]	19.67±2.51[a]	21.25±1.39[a]	23.33±0.76[a]	20.67±9.07[a]
Net protein utilization (NPU)	82.87±10.40[a]	81.57±18.44[a]	80.57±16.39[a]	74.56±14.49[a]	-	80.07±7.26[a]
Apparent digestibility (AD)	85.39±2.34[c]	89.05±1.04[bc]	89.42±0.82[bc]	89.60±1.17[b]	69.59±1.0[a]	92.63±2.57[b]
True digestibility (TD)	94.60±2.73[a]	96.63±1.06[a]	97.14±0.77[a]	97.09±1.24[a]	-	97.82±2.32[a]
Biological value (BV)	87.56±31.51[a]	84.44±19.28[a]	82.90±16.55[a]	76.73±14.36[a]	-	81.77±5.78[a]

Values are means and standard deviation (n = 6). Means in the same row with different superscript letter are significantly different from one another at 5% level ($p<0.05$). NCD = Negative control diet, RD = Reference diet (Nutrend[R]).

According to Millward *et al.*[28] and Babji *et al.*[29] PER and BV are invaluable in the determination of protein quality of food. Apart from rats adapted on diet formulated with AW_{raw} with the value of 1.86 and those of NCD that is 1.11, the PER of all the rats though statistically lower than that of RD, were above 2.0. PER measures the effectiveness of a protein in promoting animal growth. According to Indian Standard Specifications, as stated by Hei and Sarojnalini,[30] protein-rich foods should have protein efficiency ratio of at least 2.0. The PER of some plant and animal-based foods were given as follows: 1.1 (wheat), 1.2 (potato), 1.7 (beans), 2.4 (chicken and beef), 3.0 (fish meal), 3.1 (whole milk) and 3.4 (egg).[31,32] Earlier, Anyalogbu *et al.*[3] reported that the P-PER of the plant food at all levels of processing (except at AW_{135}), was higher than 1.50 below which a protein is taken to be of low or poor quality. The weight gain (BWC) of rats on all the test diets, with exception of AW_{135} varied with protein intake. The margin between the BWC of rats on RD and test diets was not correspondingly reflected in PER of the two groups, though still significant ($p<0.05$). This could be an indication that all proteins from the plant foods at all levels of processing were adequately utilized by the animals.[33] The observed decrease in PER with increased heat-contact time could be ascribed to the fact that heat accelerates millard reaction and consequently makes protein biologically unavailable.[34] Marginally, while NPU decreased, AD and TD of the sample diets increased with processing time, and apart from AD of AW_{raw}, are statistically similar ($p > 0.05$) to those of the reference diet. The biological value (BV) of rats on all the test diets were statistically similar ($p>0.05$) similar to those fed reference diet and above 70. The similarity of the BVs of the test diets to the reference diet could suggest the similarity of their protein qualities to that of the latter. BV is a measure of protein 'usability'. It measures the proportion of absorbed protein nitrogen (N) (from a food) that becomes incorporated into the proteins of the organism's body (for maintenance and growth of the organism). It is therefore a ratio of nitrogen incorporated into the body over nitrogen absorbed from food.[35] It assumes that a proportion of nitrogen absorbed from food by the body is later excreted while the remainder must have been incorporated into the proteins of the organism's body. According to Whitney and Rolfes,[36] Volken de Souza *et al.*,[37] a protein with a BV of 70% or more can support human growth and tissue maintenance as long as energy intake is adequate.

CONCLUSION

The foregoing indicates that the test sample is rich in protein that is adequately utilized and capable of supporting life. The quality indices are above recommended benchmarks and not much affected by the processing method. One could therefore conclude that the protein quality of the processed African walnut (*Plukenetia conophora*) kernel used as traditional snack in parts of Nigeria is of acceptable quality.

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