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Determination of Amino Acid Content and Protein Quality of Complementary Food Produced from Locally Available Food Materials in Ondo State, Nigeria

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ABSTRACT

Protein-energy malnutrition is increasing among children in developing countries due to low nutrient density of traditional complementary diets. Therefore, this study aimed at determining the protein quality of a complementary food produced from cooking banana fruits and bambara groundnut seeds. The cooking banana and bambara groundnut seeds are locally available in both urban and villages markets in Nigeria. The cooking bananas (CB) and bambara groundnut (BG) seeds were processed into flours using standard procedure. The flours were mixed in a ratio of 70:30 (CBR_1) and 60:40 (CBR_2) of CB and BG respectively. A commercial weaning food (Nutrend) and traditional weaning food, ogi(corn gruel), were used as control food samples. The amino acid content and protein quality of the food samples were determined using standard procedures. Glutamic acid (CBR_1 = 4.353 g / 100g, CBR_2 = 5.804 g / 100g) was the highest while cysteine (CBR_1= 0.252 g / 100g; CBR_2 = 0.336 g / 100g) was the lowest of the amino acids in the food samples. The amino acids composition increased as the percentage supplementation of bambara ground nut increased in the mixtures. The formulated food sample showed that CBR_1 and CBR_2 met 31.8% and 42.4% respectively of the recommended dietary allowance (RDA) fulfilment of essential amino acids. The biological value (BV) of CBR_2 (90.5%) was significantly high when compared with CBR_1 (75.9%) and ogi (52.4%). There was no significant difference between the BV of $CBR_2$with the BV of Nutrend (93.8%). Also, the net protein utilisation (NPU), total digestibility (TD), protein efficiency ratio (PER), feed efficiency ratio (FER) and nitrogen retention (NR) of CBR_2 were within a similar range as those for Nutrend. As for the haematological variables, there were no significant differences between those fed the formulated diets and the control samples. The rate of weight gain for the animals fed with CBR_2 food sample was higher than those fed with CBR_1 and ogi but were lower than those for animals fed with Nutrend and casein. The study established that the CBR_2 samples contained the essential amino acids needed to support infant growth and development.

INTRODUCTION

Prevalence of protein energy malnutrition (PEM) in infants after six months old is high in Africa (Plahar & Hoyle, 1991; Ojofeitimi, 1982). Protein energy malnutrition is associated with poverty and poor nutrition knowledge resulting in early weaning, delayed introduction of complementary foods, a low-protein diet and severe or

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frequent infections (Müller *et al*., 2003; Rice *et al*., 2000). The prevalence of PEM is high among infants because at this stage of development, they require higher energy and quality proteins in their diet so as to meet increasing metabolic demands. Several studies have reported that most of the weaning foods consumed by the children in many parts of developing nations are deficient in essential macronutrients and micronutrients (Levin *et al*., 1993; Pinstrup–Andersen *et al*., 1993; Brabin & Coulter, 2003; Milward & Jackson, 2004). In view of this nutritional problem, several strategies have been used to formulate weaning food (Lalude & Fashakin, 2006; Ijarotimi & Ashipa, 2006; Ijarotimi & Bakare, 2006; Ijarotimi & Ayantokun, 2006), through a combination of locally available foods that complement each other in such a way as to create a new pattern of amino acids that provide the recommended daily allowance for infants. For instance, cereals are deficient in lysine but have sufficient sulphur-containing amino acids which are the limiting factors in legumes (Tsai, Dalby & Jones,1975).

Despite all these efforts, it is evident that the formulated weaning foods currently available are not accessible to many low-income mothers due to the high cost of production and non-availability of the food materials used in the formulation (Agbede & Aletor, 2003). In order to reduce the cost of these weaning food raw supplies and thus increase their affordability, there is a need to explore the nutritional potential of alternative low cost protein sources such as bambara groundnut seeds and cooking banana fruits.

Bambara groundnuts (BG) are grown for their edible seeds which can be consumed in many ways (Linnemann, 1990; Linnemann & Azam-Ali, 1993). The seed makes a complete food as it contains sufficient quantities of protein, carbohydrate, fat and appreciable amounts of micronutrients. Studies have shown that the gross energy value of the seed is greater than that of common pulses such as cowpea and lentil, and its protein content is higher in essential amino acids particularly methionine compared to other legumes (Poulter & Caygill, 1980; Akyroyd & Doughty, 1982; Brough & Azam – Ali, 1992). Bananas (CB), consumed cooked or raw, either as still green, half ripe or ripe fruit, are one of the most significant sources of calories in the human diet. The banana is also a good source of carbohydrate, potassium and carotenoids (provitamin A). It can be fried in various ways and eaten as a side dish and is also a local fast food (Bayeri *et al.*, 1999).

Ethical approval

The study protocol was approved by the ethics committee of the School of Agriculture and Agricultural Technology, Federal University of Technology, Akure, Nigeria.

MATERIALS AND METHODS

Preparation of materials

The bambara groundnut seeds were sorted, cleaned and soaked for 24 hours in hot water for easier de-hulling. After 24 hours of soaking, the water was poured away, and the seeds were washed and allowed to drain. The seeds were roasted, using the local method, in hot fine sand at 180^{o}C, measured with a Portek thermometer, for 15 minutes. The roasted seeds were cleaned by winnowing, and were then milled and sieved through 0.4mm wire mesh screen to obtain fine (roasted) bambara groundnut flour. The cooking bananas were peeled manually, sliced into pieces and oven-dried (Galenkamp, size 3, hot box, London, UK) at 60^{o}C for 24 hours. The oven dried banana pieces were milled, sieved through 0.4mm wire mesh screen. The prepared banana and roasted Bambara groundnut flour were blended in ratios of 70: 30 and 60:40 respectively, and the blends were used for chemical analyses and in feeding the rats involved in the tests.

Amino acid analysis

Amino acid composition of samples was measured on hydrolysates using an amino acid analyser (Sykam-S7130) based on high performance liquid chromatography technique. Sample hydrolysates were prepared following the method of Moore and Stein (1963). Two hundred mg of sample were placed in a hydrolysis tube. Then 5 mL 6M HCl were added to sample into the tube, tightly closed and incubated at 110°C for 24 hours. After incubation, the solution was filtered and 200 mL of the filtrate was evaporated to dryness at 140°C for an hour. Each hydrolysate after dryness was diluted with one mL of 0.12 M, pH 2.2 citrate buffers, the same standard applied to amino acids. An aliquot of 150 μL of sample hydrolysate was injected in a cation separation column at 130°C. Ninhydrine solution and an eluent buffer (the buffer system contained sodium acetate (90%) and acetonitrile (10%)) were delivered simultaneously into a high temperature reactor coil (16 m length) at a flow rate of 0.7 ml/min. The buffer/ninhydrine mixture was heated in the reactor at 130°C for 2 minutes to accelerate chemical reaction of amino acids with ninhydrine. The products of the reaction mixture were detected at wavelengths of 570 nm and 440 nm on a dual channel photometer. The amino acid composition was calculated from the areas of standards obtained from the integrator and expressed as percentages of the total protein.

Nutritional evaluation

Experimental design

A randomised design was used in this study. Twenty-five (15 males and 10 females) weanling albino rats of the Wistar strain at approximately 4 weeks of age with an average weight of 50.5 g were used for the study. The albino rats were divided into five groups of five rats per group. The rats were individually housed in separate cubicles in a metabolic cage with facilities for separate collection of urine and faecal matter. The animals were subjected to five days of acclimatisation. Two groups of animals out of the total of five were administered with the experimental diets (containing 70:30 and 60:40 of cooking banana and roasted bambara groundnut flour respectively. The remaining three groups of animals were administered with Nutrend (a commercial weaning food), ogi (corn gruel), which is a traditional weaning food, and casein. The groups of animals were fed with the food samples and water *ad libitum* for 27 days. The body weights of the animals were measured at two-day intervals for 21 days. The total faeces and urine voided during the last 5 days of the experiment were collected, weighed and preserved. The urine collected was preserved by adding a few drops of H_2S0_4 to prevent any loss of ammonia and also to serve as a preservative agent, while the corresponding feed consumed was also recorded for nitrogen determination.

Protein quality evaluation

The nutritional qualities of the diets for the rats were evaluated using the following computations:

$$\text{Protein efficiency ratio (PER)} = \frac{\text{Gain in body weight (g)}}{\text{Protein intake (g)}}$$

$$\text{Net protein ratio (NPR)} = \text{Weight gain of test protein group} + \frac{\text{Weight loss of N – Free N- diet group}}{\text{Protein intake}}$$

$$\text{True digestibility } (TD) = \frac{Ni - (NF_1 - NF_2)}{Ni} \times 100$$

$$\text{Biological value } (BV) = \frac{Ni - (NF_1 - NF_2) - (NV_1 - NV_2)}{Ni - (NF_1 - NF_2)} \times 100$$

$$\text{Net protein utilisation } (NPU) = BV \times TD / 100.$$

$$\text{Nitrogen retention } (NR) = Ni - (F + NU)$$

$$\text{Feed efficiency ratio} = \frac{\text{Gain in body weight (g)}}{\text{Food intake of each rat (g)}}$$

where

A = desired % protein level

Y = weight of the sample produced

w = % protein level of the sample

X = expected weight to be mixed with band diet

Ni = nitrogen of animal fed with test diet

NF_1 = nitrogen executed in faeces of animal fed test diet

NF_2 = nitrogen excreted in faeces of animal fed protein-free diet.

NU_1 = nitrogen excreted in urine of animal fed test diet

NU_2 = nitrogen excreted in urine of animal fed - free diet.

Hematological evaluations

At the end of the experiment, all the rats were starved for 3 hours and weighed. Before being sacrificed, each rat was anaesthetised with chloroform inside a desiccator. Blood samples from each rat were collected into sample bottles containing a few milligram of EDTA prior to haematological analysis. The packed cell volume (PCV) was estimated by spinning about 75μl of each blood sample in heparinised capillary tubes in a haematocrit microcentrifuge for 5 minutes, and the total red blood cell (RBC) and white blood cell (WBC) counts were determined. The heart, lungs, kidneys, liver, intestine and carcass were separated, blotted free of blood, oven dried and weighed. The values were subsequently expressed in g/kg of body weight.

Statistical analysis

The data were analysed using SPSS version 15.0. The mean and standard deviations of the triplicate analyses were calculated. The analysis of variance (ANOVA) was performed to determine significant differences between the means using Duncan.

RESULTS AND DISCUSSION

The amino acid compositions of the experimental food samples are presented in Table 1. The amino acid composition of CBR_1 (70% of CB and 30% of BG) ranged between 4.353 g/100g crude protein for glutamic acid and 0.252 g/100g for cysteine; while that of CBR_2 (60% of CB and 40% of BG) ranged between 5.804 g/100g for glutamic acid and 0.336 g/100g for cysteine. In this study, it was observed that the amino acid composition of the experimental food samples increased as the percentage of bambara groundnut supplementation increased. There is evidence to show that the protein content of ogi was low (10.5 g/100g)(Oluwamukomi,Eleyinmi & Enujiugma ,2005); and that it could be improved with the addition of legumes such as soybean and cowpeas (Fashakin,Aweyefa & Furst 1986; Ijarotimi & Ashipa, 2006).

The percentage of recommended dietary allowance (RDA) of amino acids for per 100 gm of the formulated food samples is shown in Table 2. The results showed that CBR_1 sample met 31.8% of the total essential amino acids (EAA) required for children while CBR_2 food sample met 42.4% of the requirements. CBR_1 met 73% of the RDA arginine requirements while CBR_2 met 97% of the requirements but had the lowest value for methionine (15.5% for CBR_1 and 20.9% for the CBR_2) out of the total essential amino acids. The low percentage of the essential amino acids of the formulated diets compared with the RDA for EAA for children could be ascribed to the fact that the food samples were produced from plant based food materials (cooking banana fruits and bambara groundnut seeds). A number of studies have shown that the protein content of plant-based food materials is inadequate to meet the protein requirements of individuals compared with food material produced from animal sources (Akaninwor & Okechukwu, 2004; Ogunlade,Olaofe & Fadare, 2005).

Table 1. Amino acid composition (g/100g crude protein) of the formulated food samples

Amino acids	*CBR_1*	*CBR_2*
Lysine	1.503	2.004
Histidine	0.72	0.96
Arginine	1.455	1.94
Aspartic acid	2.883	3.844
Threonine	0.765	1.02
Serine	0.963	1.284
Glutamic acid	4.353	5.804
Proline	0.576	0.768
Glycine	0.897	1.196
Alanine	0.69	0.92
Cystine	0.252	0.336
Valine	0.87	1.16
Methionine	0.342	0.456
Isoleucine	1.14	1.52
Leucine	2.232	2.976
Tyrosine	0.84	1.12
Phenylalanine	1.134	1.512
Tryptophan	ND	ND

ND= Not Detected

Table 2. Percentage of RDA of amino acids met per 100g of formulated food samples

	**RDA (<3 mts.)*	*CBR_1*	*RDA% met*	*CBR_2*	*RDA% met*
Arginine	2.0	1.45	73.0	1.94	97
Histidine	2.4	0.72	30.0	0.96	40
Isoleucine	4.2	1.14	27.1	1.52	36.2
Leucine	4.8	2.23	46.5	2.98	62.1
Lysine	4.2	1.5	35.7	2.00	47.9
Methionine	2.2	0.59	15.5	0.79	20.9
Phenylalanine	2.8	1.97	40.4	2.63	53.9
Threonine	2.6	0.77	29.6	1.02	39.2
Tryptophan	1.4	ND	-	ND	-
Valine	4.2	0.87	20.7	1.16	27.6
Total EAA requirement	30.8	9.79	31.8	13.06	42.4

*Source of RDA: FAO/WHO (1973).

The nutritional quality, heamatological variables and relative organ weights of the experimental animals fed with the formulated diets, ogi, Nutrend and casein are presented in Table 3. The biological value (BV) of CBR_2 (90.5%) was significantly higher compared with that of CBR_1 (75.9%) and ogi (a corn gruel and traditional weaning food) (52.4%) ($p<0.05$). However, there were no significant differences between that of Nutrend (93.8%) and casein (97.4%). Also, the net protein utilisation (NPU), total digestibility (TD), protein efficiency ratio (PER), feed efficiency ratio (FER) and

Table 3. Nutritional quality, haematological variables and relative organ weights of rats fed formulated diets, ogi, Nutrend and casein

	CBR_1	CBR_2	Nutrend	Ogi	Casein
Nutritional quality					
BV%	75.900[c]	90.50[a]	93.80[a]	52.4[c]	97.40[a]
TD %	44.97[a]	58.13[a]	41.90[a]	42.1[c]	54.50[a]
NPU	34.20[a]	55.52[a]	42.93[a]	31.7[c]	53.47[a]
NPR	0.24[c]	0.19[b]	1.29[a]	-0.71[b]	1.94[a]
PER	0.66[c]	0.99[b]	2.09[a]	0.55[c]	2.58[a]
FER	0.06[bc]	0.10[b]	0.21[a]	0.08[b]	0.26[a]
NR	0.69[c]	1.29[ab]	1.03[ab]	0.09[d]	1.56[a]
Haematological variables					
RBC (10^6)	2.833[a]	2.933[a]	3.033[a]	2.650[a]	1.7670 [a]
WBC (mm3)	3766.67[a]	3533.33[a]	4033.33[a]	2650.00[a]	2466.67[a]
PCV %	32.333[a]	33.333 [a]	33.667[a]	30.500[a]	31.333[a]
Relative organ weights					
Heart	1.133[ab]	1.083[a]	1.083[ab]	1.350[a]	1.033[b]
Liver	3.950[c]	4.2667[ab]	5.233[a]	4.600[ab]	4.850[a]
Kidney	1.600[c]	1.533[c]	1.783[bc]	2.100[b]	2.517[a]
Intestine	13.00[b]	14.66[a]	13.42[a]	8.13[c]	10.95[c]
Carcass	46.367[c]	67.800[b]	70.169[a]	48.400[c]	75.819[a]

Note: Mean values followed by different superscripts within columns are significantly different at ($p<0.05$). Biological value (BV); Total digestibility (TD); Net protein utilisation (NPU); Protein efficiency ratio (PER); Feed efficiency ratio (FER); Nitrogen retention (NR); Red blood cell (RBC); White blood cell (WBC); Pack cell volume (PCV).

nitrogen retention (NR) of CBR_2 were significantly higher compared with CBR_1 and that of ogi ($p<0.05$); but the values were comparatively within the same range as that of Nutrend and casein. This observation was similar to other studies that reported on the nutritional qualities of food products produced from a cereal and legume combination (Onilude,Sanni & Ighalo, 1999; Ikujenlola & Fashakin, 2005; Ijarotimi & Aroge, 2005; Ijarotimi & Bakare, 2006). As for the hematological variables namely, red blood cell (RBC), white blood cell (WBC) and pack cell volume (PCV), there were no significant differences between the formulated diets and that for Nutrend, ogi and casein. However, the values obtained for RBC, PCV and WBC were generally high, thus indicating the adequacy of the formulated diets as a supplement to the commercial weaning food in a manner that promotes similar haematopoiesis. The relative organ weights of the animals fed with the formulated diets, Nutrend, ogi and casein showed no significant differences for hearts but there were significant differences between the weights of the livers, kidneys, intestines and carcasses of the experimental animals. These observations show that the formulated diets did not influence the weight of the experimental animals' organs. Hence the diets were not toxic to the animals.

The daily weight gained by the experimental animals fed the formulated diets, Nutrend and ogi are presented in Figure 1. The rate of weight gain by the animals fed with the CBR_2 food sample was higher than those fed with the CBR_1 and ogi food samples but lower than those animals fed with the Nutrend (Ijarotimi, 2006). The high daily weight gain by the animals fed with Nutrend compared with the experimental food samples might be due to the fortification of the formula with quality

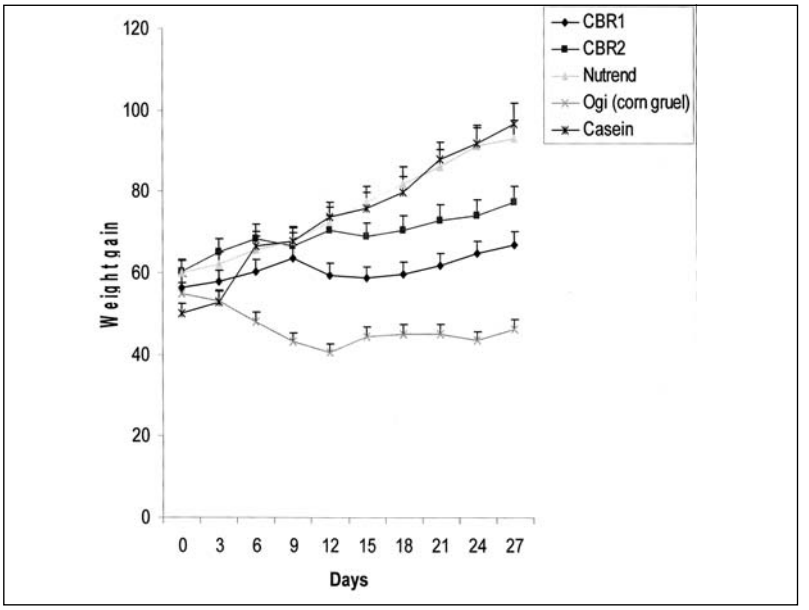

Figure 1. Weight gained by experimental rats fed with formulated diets, ogi, Nutrend and casein

protein like casein or other quality protein food materials. It is concluded from these results that the experimental food samples, particularly CBR_2, could be used as a substitute for expensive commercial weaning formula or ogi, a traditional weaning food devoid of quality protein, to alleviate the problem of protein-energy malnutrition among infants belonging to low-income families. Several studies have reported that protein-energy malnutrition is increasing in many parts of the developing world due to economic restructuring embarked upon by these developing countries and this has led to lower-purchasing power and poor feeding practices of many homes (Schofield & Ashworth, 1996; WHO, 2002; Brabin & Coulter, 2003; WHO / UNICEF, 2004; FAO, 2004; Ijarotimi & Oyeneyin, 2005)

CONCLUSION

The study established that the formulated food sample (CBR_2) containing 60% cooking banana and 40% bambara ground nut is suitable as complementary food because it contains virtually all the essential amino acids needed to support growth and development in infants.

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