Evaluation of the nutritional quality of novel weaning food processed from cooking banana and supplemented with legumes: Invitro protein digestibility and amino acid profile

F. I. Bassey*[1], M. I. Dosunmu[1], C. A. Edem[1] and I. O. Ekpenyong[1]

ABSTRACT

The nutritional quality of a novel weaning food (WF) processed from cooking banana, cowpea and peanut were evaluated by determining their amino acid profile, fatty acids, vitamins, sugars and in-vitro digestibility profile. The result revealed that some of the essential amino acids have higher values when compared with the suggested FAO/WHO amino acid requirements for the 0-1yr old namely; Histidine 32.55g and phenylanine + Tyrosine 89.06 g. While lysine was 59.28g, leucine 73.42g, tryptophan 11.70g, valine 46.19g, methionine + Cystine 25.06g, Theronine 36.86g and isoleucine 38.88g. Dietary fiber was found to be 13.05g, sugars 15.96g, protein 16.89g, Beta carotene 187IU. The calorie content was 415.59KJ while digestibility was 85.73. This also shows that the the weaning food has the potential of serving as a transition food for weaning infants in developing countries like Nigeria, where proprietary foods are out of the reach of the low income earners.

INTRODUCTION

Malnutrition continues to affect populations in developed and developing countries, resulting in increased disease and often catastrophic loss of life (Birschbach et al, 2004). In the initial months of an infant's life, the mother's milk even from a poorly nourished mother usually provides an adequate source of essential nutrients needed for active growth of the infant from birth to approximately four months of age (Agbede and Aletor, 2003). Thereafter, there is an increase in the rate of the infant's development making the mother's milk insufficient as the sole source of micro- and macro-nutrients. There is emerging evidence that diseases such as hypertension, cardiovascular diseases, respiratory diseases and diabetes are related to poor health and nutrition of the infant and mother. Baker (1994) argues that under nutrition during infancy permanently changes the body's structure, physiology and metabolism leading to coronary heart disease and stroke later in life. These studies provide convincing evidence that nutritional programming during sensitive periods in early life has long lasting effects on traits that really matter. How infants are fed appears to influence their long term development and health, thus heightening the importance of improving infant food.

Energy density of traditional cereal-based weaning foods fed to infants in developing countries is low due to their high water and low fat content (Mensa-Wilmot, et al., 2000). Nutrient requirements for infants up to 6 months of age were established from studies involving healthy infants who were exclusively breast fed by healthy mothers (FAO, 1973; WHO, 1985). The calculated energy requirements for a weaning infant ideally range from 414KJ/kg per day for a 4-5 month old to 397KJ/kg for the 8-9 month old. The recommended safe intake o f good quality protein for the infant 6-9 months of age is 1.65g/kg body weight (WHO, 1985).

The protein requirements provide nitrogen for both maintenance and growth of tissues and organs.

*Corresponding author. **Email:** fibassey@yahoo.ca
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[1]Department of Pure and Applied Chemistry University of Calabar P.M.B. 1115 Calabar, Nigeria

Over 40% of the total protein requirement should come from essential amino acids (Wardlow and Insel, 1996). There is therefore need to provide a low cost nutritious weaning supplement as a source of transition diet for the infant to ensure proper physical and mental development. The chemical score and amino acid index are widely used for screening potential protein foods (Monsoor and Yusurf, 2002). An "ideal" protein is one that contains all essential amino acids in amounts sufficient to meet requirements of the humans. The four essential amino acids that are likely to limit the protein quality in mixed human diet are: lysine, the sulfur –containing amino acids (methionine + cystine), threonine and tryptophan (WHO, 1985).

The nutritional value of protein is dependent on the digestibility as well as the availability of essential amino acids since the amino acid data only indicates a potential nutritive value of a protein that assumes amino acids of that protein are available. Since 1919, the protein efficiency ratio (PER) method which measures the ability of a protein to support growth in young, rapidly growing rats has been used in many countries because it is believed to be the best predictor of clinical tests. The short comings of PER test including lack of precision, poor reproducibility and high cost are well known. The PER and other methods were reviewed at the Airlie conference in 1980, where it was agreed that the PER should be replaced by a more appropriate and precise method (FAO/WHO, 1990).

Therefore, more rapid and less expensive in-vitro methods for assaying digestibility have been developed. The in-vitro methods for assaying digestibility all rely on the use of proteolytic enzymes to correlate with digestion of protein in-vivo. One of the best known methods was developed by Satterlee and Co-workers (Satterlee et al, 1979; Hsu et al, 1977) where the rate of enzymatic digestion is calculated from the pH drop following a 10min incubation with trypsin and chymotrypsin and intestinal peptidase at 37°C (Hsu et al, 1977) or often an additional 10 min incubation with microbial protease at 55°C (Satterlee, et al, 1979). The multi-enzyme system is preferred as a result of its similarity to the actual digestion environment in-vivo (Wong and Cheung, 2001). The objectives of this study were to evaluate the nutritional quality and in vitro digestibility of a novel weaning food processed from cooking and supplemented with legumes.

MATERIALS AND METHODS

Lightly roasted peanut, ripe cooking banana and black-eyed cowpea were purchased from Tara Foods in Albany, International Farmer's Market in Lake City and Twelve Baskets in Mableton respectively, all in Georgia, U.S.A and processed into weaning food according to the method described by Bassey (2005) and stored at -18°C.

Amino acid determination

Equivalent weight of 100mg protein was placed in hydrolysis tubes to which 0.5ml internal standard solution (norleucine, 2.5mM in 0.1MHCl) was added. Dithiodipropanoic acid (DTDPA) 3ml was dissolved in 0.2M NaOH and 5ml of 12M HCl containing 2.5% phenol were added to the hydrolysis tube; finally 1.5 ml of deionized water was added. Hydrolysis was then carried out by deareting the hydrolysis tube through three circles of vacuum with argon purging and finally leaving it under vacuum. The hydrolysis tube was then placed in a heating block at 145°C for 75 min after which it was removed and allowed to cool. Methanol (2ml) was then added to 1ml of the hydrosylate in an evaporator tube and evaporated under nitrogen to dryness. The sample was reconstituted with 25ml de-ionized water in a volumetric flask and filtered through a 0.2micron millipore filter and stored in vials for use.

Conversion of the amino acid in the standard to a highly stable derivative was then carried out by transferring 10µL of the filtrate into a test tube and adding 70µL of AccQ flour borate buffer (vortexed) and 20µL AccQ flour reagent; the mixture was immediately vortexed and allowed to settle for a minute and then transferred into a low volume vial inserted in a 4mL vial. Heating was done for 10 min at 55°C and the mixture separated by HPLC.

In vitro Protein digestibility

The pH drop method by Hsu et al (1977) as modified by Satterlee et al (1979); El-Hady and Habiba (2003) and Reyes-Moreno et al, (2003) was used. Two water baths were pre-set to 37°C and 55°C respectively. The pH of an aqueous suspension (50 ml) of sample containg 6.25mg protein/ml in distilled water was adjusted to pH 8.0 with 0.1M HCl while stirring in the water bath maintained at 37°C. A 100ml multienzyme solution prepared from 1.6mg trypsin, 3.1mg chymotrypsin and 1.3mg peptide/ml was kept in an ice bath and its pH adjusted to 8.0 with 0.1M HCl. Five milliliters of the multienzyme solution was then added to the sample suspension with constant stirring at 37°C. A rapid decrease in pH occurred due to the freezing of the amino acid carboxyl groups from the protein chain by

the protolytic enzymes. At exactly 10 min from the time the solution was added, the pH was measured using a pH meter. In-vitro protein digestibility was calculated using equation 3.8 below reported by Hsu et al (1977).

PD= 210.464 – 18.103X (1)

where PD = protein digestibility and X = pH 10 min after addition of enzymes to protein suspension.

The sample was then transferred to the water bath maintained at 55°C and at the end of 19 min of reaction, the tubes were transferred back to the water bath maintained at 37°C and the final pH measurement carried out at the end of exactly 20 min from the start of the reaction and a second protein digestibility calculated using equation 3.9 by Satterlee et al (1979) where,

%PD=234.8422.56X (2)

Where X is the pH of the suspension after 20 min hydrolysis of the protein.

Vitamins analyses

Vitamins were analyzed using an HPLC method described by Lee et al (2000). Ascorbic acid was extracted with metaphosphoric acid and acetic acid and quantified by fluorometric analysis using Official Method of AOAC (1995). All samples were analyzed in triplicate.

Analysis of sugars

Sugars were analyzed by HPLC as described by Linden and Hurst 1996.

Fatty acid analyses

Fatty acid was determined using gas-liquid chromatography with flame ionization detection (GLC-FID)/capillary column based on the method used by Oliveira, Alves and Ferreira (2001).

Mineral ananlysis

Calcium, Sodium and Iron were analysed using Official Method of AOAC (2000). All samples were analyzed in triplicate

Statistical Analysis

Data were analyzed statistically with the aid of the Statistics Analysis Software (SAS, 1990).

RESULTS AND DISCUSSION

Table 1 shows the essential amino acid profile of the formulation and that proposed by FAO/WHO (1989) for 0 to 1yr old and the pre-school age group (2 to 5yr old) and the percentage score as predicted by ESHA.. Mensa-Wilmot et al (2001) in their study on protein quality evaluation of cowpea-based extrusion cooked cereal/legume weaning mixtures recorded amino acid profil within the range of 26-31, 31-36, 71-76, 47-52, 22-23, 95-108, 32-36 and 39-43, for histidine, isoleucine, leucine, Methionine + Cystine, Phenylanine + Tyrosin, Tryptophan and valine respectively.

The formulation was observed to have protein digestibility of 85.73% (table 2).

Mensa-Wilmot et al (2001) in their study on protein quality evaluation of cowpea-based extrusion cooked cereal/legume weaning mixtures recorded digestibility ranging from 87.74-92.08%. Wong and Cheung (2001) in their work on the in-vitro digestibility of some sub-tropical red and green sea weeds obtained digestibility values in the range 88.7-88.9%. In their study Khokar and Chanhan (1986), Prinyawiwatkul et al. (1996) and Phillips et al. (2003) showed that soaking and cooking reduce and eliminate the anti nutritional factors in legumes and improve digestibility. Dry heat treatment was also shown to inhibit the activity of trypsin inhibitors (Marquez et al, 1998). The findings of Monsoor and Yusuf (2002) in their work on in vitro protein digestibility of some legumes showed that heat has no adverse effect on the digestibility of legume protein under model condition and that wet heat treatment increases digestibility significantly and thus improves protein quality. Opstvedt et al. (1984) found a linear decrease in the content of –SH (sulfhydryl groups) and a concomitant decrease in the content of S-S bonds when rainbow trout was heated at increasing temperature from 50-115°C. The impact of disulphide bond formation on protein utilization is not fully known, but some experimental data indicate that it may reduce protein utilization, (Opstvedt et al., 1984). Mauron (1984) reported that protein digestibility was reduced as a result of complex chemical (cross linking) reactions such as protein interactions or protein-fat interactions when food was boiled at high temperatures.

Table 2.Invitro digestibility of novel weaning compared with that of two proprietory products

Sample ID	Digestibility
WF	85.73B
[a]RB	77.10C
[b]OB	95.31A

Values not followed by the same letter (Duncan grouping) are significantly different

($P \geq 0.05$) as determined by Fisher's least significant difference test (LSD).

[a]Rice with banana

[b]Oates with banana

Table 1. Essential amino acid content of weaning food (WF).

Essential amino acid	Weaning food (mg/g)	FAO/WHO* 0-1yr	FAO/WHO* 2-5yr	% Score as Predicted by ESHA
Histidine	32.55	26	19	171
Isoleucine	38.88	46	28	139
Leucine	73.42	93	66	111
Lysine	59.28	66	58	102
Methionine + Cystine	25.06	42	25	101
Phenylanine + Tyrosine	89.06	72	63	141
Theronine	36.86	43	34	108
Tryptophan	11.70		11	106
Valine	46.19	55	35	132

* = suggested amino acid pattern of requirement (FAO/WHO,1989)

Table 3. Sugar, mineral and vitamin contents of weaning food

Label analytes	100 grams	Analytical data per 100g	Analytical data per serving
Sodium	(mg)	9.1(mg)	9.1(mg)
Calcium	(mg)	55.9(mg)	55.9(mg)
Iron	(mg)	3.0(mg)	3.0(mg)
Vitamin C (mg)	(mg)	1.54 (mg)	1.54 (mg)
Vitamin E (iu)	(iu)	0.84(iu)	0.84(iu)
Vitamin A (iu)	(iu)	187(iu)	187(iu)
Beta carotene	(iu)	187	
Vitamin A % Beta carotene	(iu)	100	

Table 3 shows the mineral and vitamin contents of the formulated food. The iron, sodium and calcium contents of the developed weaning food were in the order; 3.0, 9.1 and 55.9mg/100g for iron, sodium and calcium respectively. Shulk et al. (1986) recorde1.1% calcium and 14mg of iron in 100mg of developed weaning food Soylac. Chandrasekhar et al. (1988) in their weaning mixture reported 290mg calcium and 7.6mg iron respectively. Dahiya and Kapoor (1993) also reported iron within a range of 16.7-17.7mg/100g,, calcium, 153-184mg/100g respectively in four home processed weaning foods based on locally available foods. Nago et al.,(1998) reported 5.mg calcium, 3.6mg iron and 2.8mg sodium for a Beninese traditional ogi. The mineral and vitamin contents of the processed weaning food may be improved by fortification.

It maybe concluded that the weaning food when supplemented with minerals and vitamins has the potential of providing nutritious transition supplement for weaning infant in developing countries with special reference to Nigeria where proprietary foods are out of the reach of a majority of the citizens who are low income earners.

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