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The Analyses Of Amino Acid, Fatty Acid And Mineral In A Legume-Cereal Based Complementary Food Blend Used In Jos, Nigeria

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Abstract

Background: Inadequate nutrient intake is an important issue in Nigeria, where under nutrition is common among infants of weaning age and young children. The formulation of complementary foods using available/affordable staple food commodities is one approach that has been recommended to reduce malnutrition in children. This approach is achievable because the foods are intended to be prepared at household level. Various communities are endowed with staple foodstuff that can be utilized to formulate foods that are suitable to their settings. Where the food does not contain sufficient of any nutrient, especially the essential ones, correction may be needed.

Objective: The study sought to know through analyses, the amino acid, fatty acid and mineral element composition of the composite blend of a legume-cereal complementary food (SBG-C), and to ascertain its potential in meeting the recommended daily nutrient intake of infants.

Methods: Gas-Liquid chromatography, Reversed-Phase high performance liquid chromatography and atomic emission spectrophotometer were used to determine fatty acid, amino acid and mineral content of the blend respectively. Results were compared with WHO recommendations.

Results: The blend SBG-C contained all the essential amino acids, which also compared favourably with the “WHO ideal” protein. The fat content of the blend stood at 2.61g/g dm. Total saturated and unsaturated fatty acids were 16.57% and 83.43% respectively. Linoleic acid (18:2n-6) was the most abundant unsaturated fatty acid, while palmitic acid (16;0) was the most abundant saturated acid. A wide range of mineral elements were detected. K,P,Mg,and Ca were the most notable followed by Fe,Na,Al,Zn and Mn.

Conclusion: The complementary food SBG-C contains substantial amounts of essential nutrients, which would significantly supplement breast milk. Frequent feeding may however be necessary to ensure optimal growth and development in infants.

INTRODUCTION

Complementary foods are semisolid or solid foods which are introduced to the diets of breast or formular-fed infants during transition to an adult diet .Sometimes such foods are also used to rehabilitated undernourished children especially those recovering from illness.

In most developing countries, where inadequate intake of nutrients has continued to be the major cause of under-nutrition, and the resultant deficiency disorders in children, appropriate feeding practices play a vital role in ensuring that the infant gets the right and adequate food at the right time. This should not only be seen as a stepping stone to the adult (Family) diet, but also as a necessary source of additional nutrients required for optimal growth and development [1]

In Nigeria, most rural and urban mothers practice breastfeeding, some exclusive, others mix(breast and bottle feeding) [2].Although complementary feeding usually begins at 4-6 months, it is not uncommon for children to be breast-fed past two years [1,2],which is the best recommended practice. The type complementary food used may vary depending on the available foodstuff and resources. For most rural and poor urban mothers, it involves the use of traditional home-made semisolid porridges prepared from staple cereals, tubers, legumes and condiments [3,4,5]. Due to time-related constraints, inadequate and inappropriate processing and preparation of home-made weaning foods often result to stiff consistency and bulk that combine to offer a low cost filling meal that lack adequate nutrient value[1]. It is however believed that well formulated, processed and hygienically prepared home-based diets remain the most practicable option and practice that would ensure availability and affordability by the low-income “bracket”[4,5].To reduce time-related constraints faced by mothers, it is important that foods are prepared in forms that can increase the shelf-life and reduce the frequency of preparation.

The analyses of locally formulated complementary foods do not often pay attention to essential nutrients that must be supplied in the diet. The aim of this study therefore was to formulate a composite powdered blend and analyses carried out on the essential fatty acids, amino acids and mineral elements, to ascertain the adequacy the nutrients in meeting the needs of infants and young children.

METHODS

PREPARATION OF COMPOSITE BLEND OF SBG-C.

The food commodities used in the formulation of composite SBG-C are; soyabeans (Glycine max), groundnut (Arachis hypogea), acha (Digitaria exalis) and crayfish (Macrobracium spp). The food commodities were purchased from local markets in Jos, Nigeria, and processed using methods employed by the communities .Soyabeans was soaked in tap water for 3hours, washed to remove testa, boiled for 20minutes, sun-dried for 48hrs and roasted slightly. Groundnut was washed, sun-dried and roasted slightly. Acha and crayfish were simply cleaned to remove sand particles.

The processed foodstuff were mixed in the ratio 60:20:10:10 %w/w of acha:soyabeans:groundnut:crayfish. The mixture was ground to a smooth homogenous powder with the aid of a stainless steel mill, and dried under vacuum at room temperature until a constant weight was obtained. Sample was maintained under the same conditions until required for analysis. All analysis were carried out in triplicates.

AMINO ACID ANALYSIS.

Five milligram of the powdered sample was weighed and placed in 2ml ampoules, to which the internal standard (norleucine) and 0.45 ml of 6N HCl were added. Norleucine was used as internal standard because it is an amino acid not commonly found in proteins. The ampoules were evacuated, sealed and placed in an oven for 24 hrs at 110 ° C.

After hydrolysis,20μL aliquots of the hydrolysates were dried, mixed with 10μL of redry solution (ethanol:water:triethylamine,2:2:1v/v), dried again, and finally derivatized with 20uLphenylisothiocynidereagent(ethanol:water:triethylamine:phenylisothiocyanide,7:1:1:1v/v) for 20min at room temperature [6].Excess reagent was removed with the aid of a vacuum at room temperature. Derivetized samples were dissolved in 0.1ml of 0.14 M sodium acetate that had been adjusted to pH 6.4 with dilute aceti acid.A $20_{\mu L}$ aliquot was injected onto the column. Quantization of amino acid was performed using a Waters C18 column (3.5x150 mm)with gradient conditions as described elsewhere[7]. Derivatized amino acids were eluted from the column with increasing concentrations of acetonitrile. The eluate was monitored at 254 nm and the areas under the peaks were used to calculate the concentrations of the unknowns using the Pierce Standard H amino acid calibration mixture (Rockford,IL). Norleucine was the internal standard used in all amino acid determinations. A sample of egg white lysozyme analyzed in duplicate, served as the control protein.

Samples intended for the determination of cysteine were first oxidized with performic acid (80% formic acid and 30% hydrogen peroxide,9:1) for 18 hrs at room temperature[6].The oxidizing reagent was removed with the aid of an evaporative centrifuge and the samples were with 6N HCl as described above.

The tryptophan content was determined in a separate analysis. The weighed samples were placed in polypropylene tubes and after the addition of the internal standard (norleucine), they were hydrolyzed in 4.67M KOH containing 1% w/v thiodiglycol for 18hrs at 110 ° C.After hydrolysis the KOH was neutralized with 2.4M perchloric acid, and the supernatant was adjusted pH 3.0 with acetic acid. A 20μL aliquot of the hydrolysed sample was subjected to derivatization as described above. The solution of amino acid standard was supplemented with tryptophan. Quality assurance for the tryptophan determination was obtained by demonstrating that the method yielded the correct number of tryptophan residues for egg white lysozyme.Tryptophan analysis was performed using a Waters C18 reversed phase column (3.9 x150 mm) (Waters Milford,MA) and the solvents and gradient conditions were as described by Hariharan et al [8].Use of this elution protocol was necessary in order to adequately separate tryptophan from ornithine which results from the alkaline hydrolysis of arginine.

Fatty acid analysis. The dried powdered blend of SBG-C was extracted with chloroform:methanol (2:1v/v) and solid non-liquid material was removed by filtration. The total extracted lipid material was recovered after solvent removal in a stream of nitrogen. The samples were the redissolved in anhydrous chloroform/methanol (19:1 v/v),and clarified by centrifugation at 10,000 x g for 10min.Tranmethylation was performed using 14% (w/v) boron triflouride(BF_3) in methanol[9].Fifty nanograms of heptadecanoic acid (internal standard) and 1ml aliquot of each sample were transferred to a 15ml Teflon-lined screw-cap tube. After removal of solvent by nitrogen gassing, the samples were mixed with 0.5 ml of BF_3 reagent (14% w/v),placed in warm bath at 100 ° C for 30 min and cooled. After the addition of saline solution, the transmethylated fatty acids were extracted into hexane. A calibration mixture of fatty acid standards was processed in parallel.

Aliquots of the hexane phase were analyzed by gas chromatography. Fatty acids were separated and quantified using a Hewlett-Packard gas chromatograph (5890 Series II) equipped with a flame-ionization detector. Two µl aliquot of the hexane phase were injected in split-mode onto a fused-silica capillary column (Omegawax:30m x 0.32mm ID, Supleco, Bellefonte, PA).The injector temperature was set at 200 ° C,detector at 230 ° C,oven at 120 ° C initially, then 120-205 ° C for 18min.The carrier gas was helium and the flow rate was approximately 50 cm/sec. Electronic pressure control in the constant flow mode was used.The internal standard (heptadecanoic acid,C17:0) and calibration standards (NuCheck, Elysian, MN) were used for quantitation of fatty acids in the lipid extracts. The fatty acids reported represent the average of three determinations.

MINERAL ANALYSIS.

Three replicate aliquots (50-500 mg) from the dried powdered complementary food SBG-C were weighed, then wet-ashed by refluxing over night with 15 ml of concentrated HNO_3 and 2.0 ml of 70% $HClO_4$ at 150 ° C.The samples were dried at 120 ° C and the residues were redissolved in 10 ml of 4.0N HNO_3-1%$HClO_4$ solution. The mineral content of each sample solution was determined by inductively coupled argon plasma atomic emission spectroscopy (ICP-AES,Jarrel-Ash) as described elsewhere[2,4].The mineral content of the samples were quantified against standard solutions of known concentrations which were analyzed concurrently.

RESULTS

Amino acid content. The amino acid content of the complementary food SBG-C is shown in Table 1. All the essential amino acids as well as the non essential ones were detected. The total weight of (mg/g dm) of the amino acids was 265. Mean % of total showed that glutamic acid (17.2%), aspartic acid (10.8%), leucine (8.90%),and arginine(7.09%), were reasonably present, while cysteine (1.43%) and methionine (1.15%) were found to be the least.

Figure 1

Table 1:Amino acid content of SBG-C

Amino acid	Mean(SD) (mg/g dm) (n=3)	Mean (% of total)
cysteine	3.79 (0.04)	1.43
Aspartic acid	28.6 (0.31)	10.8
Glutamic acid	45.7 (1.43)	17.3
Serine	13.8 (1.10)	5.20
Glycine	10.4 (0.19)	3.93
Histidine	8.37 (0.11)	3.16
Arginine	18.8 (0.05)	7.09
Threonine	10.6 (0.56)	4.01
Alanine	11.9 (0.02)	4.49
Proline	17.1 (0.27)	6.44
Tyrosine	8.74 (0.09)	3.30
Valine	13.2 (0.09)	4.98
Methionine	4.00 (0.02)	1.51
Isoleucine	12.1 (0.08)	4.55
Leucine	23.7 (0.11)	8.92
Phynylalnine	14.9 (0.24)	5.61
Tryptophan	5.69 (0.26)	2.15
Lysine	13.8 (0.21)	5.20
Total	265	

Table 2 shows the essential amino acid content compared to “WHO Ideal” amino acids. All the essential amino acids detected in SBG-C blend compared favorably with the WHO ideal.

Figure 2

Table 2: Essential amino acid content of complementary food SBG-C compared to the WHO "ideal protein "

Amino acid	Blend of SBG-C: % of total[b]	Blend of SBG-C: % of total / % in "ideal protein"	WHO "ideal protein"
Isoleucine	4.6	164	2.8
Leucine	8.9	135	6.6
Lysine	5.2	90	5.8
Methionine + Cystiene	2.9	118	2.5
Phenylalanine+Tyrosine	8.9	141	6.3
Threonine	4.0	118	3.4
Tryptophan	2.2	195	1.1
Valine	5.0	143	3.5

[a]WHO [10]
[b]Total protein,265 mg/g dry weight

FATTY ACID CONTENT OF SBG-C

The fatty acids detected in the composite blend of SBG-C are as shown in table 3.The percentage of each fatty acid in the total is also shown. Unsaturated fatty acids were the most present (83.3%). Linoleic acid 18:2n-6 was the most abundant (45.7% of total), followed by oleic acid 18:1n-9 (31.9 % of total). Total saturated represented only 16.7%, with palmitic acid 16:0 as the most abundant (11.2% of total), followed by stearic acid 18:0(3.75% of total).Dihomo-linoleic 20:2n-6 (0.05% of total) was the least unsaturated, while myristic 14:0 (0.06% of total) was the least saturated fatty acid.

Figure 3

Table 3: Total lipid and fatty acid content of SBG-C blend

Fatty acid		Content in SBG-C Mean (SD) (mg/g dm)	% of total
Myristic	14:0	1.61 (0.49)	0.06
Myristoleic	14:1	0.30 (0.0)	0.01
Pentadecanoic	15:0	0.43 (0.03)	0.02
Palmitic	16:0	293 (41.0)	11.2
Palmitoleic	16:1	3.58 (0.31)	0.14
Stearic	18:0	97.9 (4.15)	3.75
Oleic	18:1n-9	832 (31.4)	31.9
Moroctic	18:1n-7	28.3 (1.77)	1.08
Linoleic	18:2n-6	1190 (68.9)	45.7
α-Linolenic	18:3n-3	106 (9.10)	4.06
Arachidic	20:0	15.7 (0.14)	0.6
Gadoleic	20:1n-9	10.8 (0.18)	0.4
Dihomo-linoleic	20:2n-6	1.21 (0.29)	0.05
Behenic	22:0	17.4 (0.64)	0.67
Erucic	22:1n-9	2.46 (0.18)	0.09
Lignoceric	24:0	8.45 (0.64)	0.32
Total lipid		2610	

The comparison of fatty acid composition to WHO/FAO recommendations, and the calculated daily intake of essential fatty acids compared to RDV are shown in tables 4&5 respectively. Total saturated (16.7%) and total unsaturated (83.43%) as well as ratio of unsaturated: saturated (15:1), well exceeded the WHO/FAO recommendations of 3.5,52.9-44.8,1.16-0.84 respectively. The ratio of 18:2n-6 : 18:3n-3 was 11:1 as compared to 5;1-10:1 recommended by WHO/FAO. The daily consumption of 65g of SBG-C would provide 77.35g of linoleic acid and 7.15g of linolenic acid, which are higher than RDV values of 0.5 and 0.05-0.1g/day respectively.

Figure 4

Table 4: Essential fatty acid content of SBG-C blend compared to WHO/FAO recommendations

	Analysed values (% w/w)	WHO/FAO [a] Recommendation
Total saturated	16.57	3.5
Total unsaturated	83.43	44.8-52.4
Unsatd:std	15:1	0.84-1.16
18:2n-6	45.7	2.0
18:3n-3	4.06	0.8
18:2n-6:18:3n-3	11.1	5:1-10:1

[a] [1,11,12]

Figure 5

Table 5:Estimated daily intake of essential fatty acid(EFF) in SBG-C blend (g/65g) compared to RDV (g/day)

EFF	Estimated value in 65g SBG-C	RDV
Linoleic 18:2n-6	77.35	0.5
Linolenic 18:3n-3	7.15	0.05-0.1

[a] the dry weight estimate of the daily intake of weaning food by a six mouth old infant in Jos, Nigeria [1]
[b]Recommended daily values [1,13]

Mineral element composition of SBG-C blend

The results in table 6 show that K,Ca,Mg and P were the most abundant mineral elements (6220,1330,1370,3330 μg/g dm respectively). The concentrations of Fe,Zn and Mn were also reasonably high(107.3,35.9,16.3 μg/g dm respectively). Elements such as Pb,Cd and Cu, considered to be heavy metals were also detected in minute quantities.

Figure 6

Table 6: Mineral content of SBG-C blend

Mineral	Mean (SD) (µg/g dm) (n=3)
Aluminium Al	44.3 (20.9)
Arsenic As	0.29 (0.28)
Barium Ba	12.3 (0.35)
Beryllium Be	0.01 (0.001)
Calcium Ca	1330 (100)
Cadmium Cd	0.008 (0.01)
Cobalt Co	0.04 (0.01)
Chromium Cr	1.40 (0.97)
Copper Cu	8.73 (2.07)
Iron Fe	107.3 (4.73)
Potassium K	6220 (118.5)
Lithium Li	0.05 (0.02)
Magnesium Mg	1370 (56.9)
Manganese Mn	16.3 (0.57)
Molybdenum Mo	4.13 (0.27)
Sodium Na	68.7 (1.19)
Nickel Ni	1.03 (0.02)
Phosphorus P	3330 (125)
Lead Pb	0.32 (0.09)
Strontium Sr	8.64 (0.20)
Titanium Ti	0.19 (0.16)
Thallium Tl	0.35 (0.33)
Vanadium V	0.04 (0.01)
Yttrium Y	0.02 (0.01)
Zinc Zn	35.9 (1.51)
Zirconium Zr	0.22 (0.38)

DISCUSSION

Inadequate nutrient intake is an important issue in Nigeria where under nutrition is common in infants and young children of weaning age. The development and use of nutritious complementary foods from available and affordable food commodities, is one approach that is being encouraged as part of effort to reduce morbidity and mortality in children. This is achievable because such foods are intended to be prepared at household level. The preparation and use of home-based foods in Nigeria have been observed to be deficient in some essential nutrients [1,2,5,14]. In circumstances where the diet does not contain sufficient nutrients, correction may be needed particularly micronutrients, essential amino and fatty acids.

The formulation and analyses of the complementary food SBG-C afforded the researchers to determine both in qualitative and quantitative terms, the adequacy of the blend to meet the recommended dietary requirements for essential amino acids, fatty acids and mineral elements in infants and young children.

Amino acids. The quantity and quality of protein is an important consideration in child nutrition as both are required for optimal growth and development. Protein quality is a function of the amino acids present. The essential amino acids which can only be provided in the diet must be adequate for normal protein turnover in the body. The protein and amino acids contents of the analyzed blend compared favorably with the WHO “ideal protein” for children. Only lysine was observed to score slightly below (5.2) the ideal level (5.8).The results tend to agree with the assertion that high protein content of legumes increases the protein content of cereal-based complementary foods and supplement the deficient amino acids [11].

After ascertaining that the complementary food contain all the essential amino acids, the researchers sought to see in specific terms, the total amino acids (in grams) which represents the amount of protein in the diet by comparing with RDA for a six month old infant which is put at 13-14 g per day [19].The computed result shows that 65g(dry weight estimate of the daily intake of weaning food by a six-month old infant in the study area [1],of SBG-C would provide 17.2g of protein per day, thereby meeting the RDA criteria for infants. The digestibility of the protein remains to be established.

Fatty acids. The blend of SBG-C contains appreciable amounts of both saturated and unsaturated (MUFA&PUFA) acids. The World Health Organization/Food and Agricultural Organization recommend that during weaning, and at least until two years of age, the child's diet should contain 30-40% from fat and provide similar levels of essential fatty acids as are found in breast milk. The fatty acid composition of the blend was therefore compared to WHO/FAO recommendations based on the average fatty acid contents of human milk in Europe and Africa.[12,13].It was found that the blend would provide 2.61 g/g dm and the essential fatty acids linoliec 18:2n-6(45.7% of total) and linolenic 18:3n-3 (4.06% of total).The saturated and unsaturated as well as ratio of saturated:unsaturated well exceeded the WHO/FAO recommendations. Similar observation was made in a soyabean-based weaning food studied in the same locality [1] The ratio of std:unstd fatty acid is used to express the health value of a fat, The balance is important with respect to coronary heart disease [19].This is exemplified by the fact that saturated acids elevate serum cholesterol and LDL, while unsaturated lowers same [18] Additionally, essential fatty acids are vital for normal fetal development and infant growth, since Infants generally utilized the EFF to synthesize other long chain PUFA used extensively for the central nervous system membrane and photoreceptor cell development during the first year of life [1]

Mineral elements. With regards to mineral content, the complementary food SBG-C contained appreciable amounts of the mineral Fe,Mn,Mg,Cu , Cr andMo and comparatively low in Ca, P and Zn. Similar inadequacies of some minerals have been observed in various home-made formulations in Nigeria [1,5,15].In this regard, supplementation of household complementary foods with mineral-dense foodstuff and condiments have been suggested to include, baobab fruit pulp which is rich in protein, iron and calcium[16] ,and crushed giant grasshopper rich in calcium, phosphorus and zinc [17].

However, since complementary foods are intended to supplement breast milk, it is hoped that the deficient minerals in the blend would probably be met by the mother's milk. Importantly too, it is not enough to have sufficient amounts of minerals in complementary foods, it is more important to have them in a form in which they are easily digestible and available to the body. This is an especially pertinent issue in a country such as Nigeria where most of the diets are of plant sources with inherent antinutritional factors which lower the utilization of nutrients unless they are processed. Breast milk provides the best forms of minerals and other nutrients to the infant.This are why it is recommended that breastfeeding continues, while food supplements. Minerals and vitamins are essential for normal growth and development, utilization of macronutrients, maintenance of an adequate defense against infectious diseases, and for many other metabolic and physiological functions .The bioavailability of the minerals in this study is yet to be established.

It is worth noting that the powdered blend of SBG-C is prepared for consumption by the addition of boiled water to make a liquid porridge, which results in an easily digested and feeling meal for the infant but which contains a lower nutrient density than the dry matter. So one would not assume that the nutrient content of the studied powdered blend of SBG-C, is an accurate predictor of the nutrients actually being consumed and utilized by the child. In this regard, the researchers recommend that feeding with the prepared porridge of SBG-C blend be more frequent and the period of breast feeding be extended to at least two years.

Conclusion. The study has shown that the formulated powdered complementary diet which can be prepared at household level, contains appreciable amounts of essential amino acids, fatty acids and minerals. The blend would however require some level of supplementation with mineral-dense sources, while breast feeding is encouraged for a longer period with improved maternal diet. It is anticipated that data from this study would make contribution to databank for locally formulated complementary foods used in Africa and Nigeria in particular. It is hoped that dissemination of such knowledge to the benefiting communities would improve child care and feeding practices.

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