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Original Article

Fatty acid, amino acid and trace mineral analysis of three complementary foods from Jos, Nigeria[☆]

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Abstract

Complementary foods (CF), commonly known as weaning foods, are semi-solid or solid foods that are used to transition infants from breast milk to an adult diet. Their nutritional content is important to the growth and development of children, particularly in developing countries such as Nigeria. In a previous study five CF produced in Jos, Nigeria were analyzed for their nutritional content. Based on those findings, three new CF were formulated in an effort to improve the nutritive value. The new formulations (second-generation CF) were analyzed for fatty acid (FA), amino acid, and mineral and trace element content. The results were compared to those of the most nutritious CF previously analyzed (designated Soy). The total FA content of all three second-generation CF (3.89–20.8 mg/g) was lower than the first-generation Soy

Abbreviations: ABR, acha, bambara nut, rice; AI, adequate intake; ATC, aya, tamba, carrots; CF, complementary foods; DHA, docosahexaenoic acid; DRI, dietary reference intakes; EAR, estimated average requirement; EFA, essential fatty acids; FA, fatty acid(s); JUTH, Jos University Teaching Hospital; MMSG, maize, millet, sorghum, groundnuts; RDA, recommended dietary allowances; UL, tolerable upper intake level; WF, weaning food(s); WHO, World Health Organization.

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mixture (105 mg/g). The content of linoleic and α -linolenic acids among the second-generation CF (1.64–10.1 and 0.084–0.63 mg/g, respectively) was also lower than the Soy CF (59.7 and 7.46 mg/g). The second-generation CF all had higher iron content than Soy (138–288 versus 98.1 μ g/g). The amounts of magnesium (1030–1733 versus 2255 μ g/g), phosphorus (2237–3830 versus 5685 μ g/g), and zinc (28.9–37.9 versus 54.8 μ g/g) in the second-generation CF were lower than in Soy. The second-generation CF also had lower protein content than Soy (66–197 versus 355 mg/g). Overall, the new second-generation CF had a lower nutritive content than the original Soy CF.

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1. Introduction

During the first 6 months of life, breast milk is the preferred nutrient source for the infant since it contains easily digestible fatty acids and proteins for growth, while also providing protection against disease, especially diarrhea (Werk and Alpert, 1998; Hautvast et al., 1999). Breastfeeding not only decreases an infant's susceptibility to gastrointestinal disease, but also frees the family from the financial burden of buying formula milk and reduces a mother's risk of breast cancer (Werk and Alpert, 1998). However, after 6 months of age, the energy and nutrient requirements of the infant exceed what can be supplied by breast milk alone (Werk and Alpert, 1998).

In Nigeria, as in most other sub-Saharan countries, breastfeeding is practiced by nearly all women in both rural and urban areas (Bentley et al., 1991; Jansen, 1992). In that part of the world, complementary feeding is usually begun between 3 and 6 months of age, but the length of time a mother will breastfeed has been shown to vary from 1 to 2 years depending on socio-economic factors (Jansen, 1992). Complementary foods (CF), also known as weaning foods (WF), are semi-solid or solid foods that are introduced to the diet of a breast- or formula-fed infant during the transition to an adult diet. They are also used as an easily digestible supplemental source of nutrients for children or adults, especially during recovery from disease.

The recommendations for complementary feeding from the American Academy of Pediatrics include exclusive breastfeeding for the first 4–6 months of age, introduction of solid foods at 4–6 months of age, and continued breastfeeding to the first birthday and beyond if possible (Kleinman, 1998). The World Health Organization (WHO) recommends exclusive breastfeeding for 6 months, with introduction of CF and continued breastfeeding thereafter (World Health Organization/Food and Health Organization, 1995).

The timely introduction of properly formulated and prepared CF is necessary for the growth and survival of infants, especially in the developing world (Jansen, 1992). In Nigeria, the introduction of CF usually involves use of a semi-liquid porridge prepared locally by the mother from staple cereals or tubers (Bentley et al., 1991; Nout, 1993; Gupta, Esrey, Oni, & Brown, 1993). Legumes such as soybeans, bambara nut and groundnut are often used to complement the high cereal content. However, traditional CFs rarely fulfill the nutritional needs of the African infant. The low energy and low nutrient density, stiff consistency and high volume of homemade porridges combine to offer a filling meal at low cost, but one that usually lacks adequate nutritive value.

In the Jos Plateau of Nigeria there are five locally prepared commercially available CF that are within economic reach of the general population. These CF were analyzed previously (Fernandez et al., 2002). The results of the analysis revealed the soybean-based CF was more nutritious than the rice-based CF. Of the five CF analyzed it had the highest amount essential fatty acids and total protein.

In an effort to improve the nutritive quality, specifically to increase the mineral content, of the CF in the region, one of the authors of the previous study formulated three new CF. The goal of the present study was to assess the nutrient content of the three new CF. In particular, the qualitative and quantitative aspects of the fatty acid, amino acid and mineral composition were analyzed. These results were compared to the most nutritious CF of the previous study (Soy). Also, the estimated daily total nutrient intake of 65 g of the new CF by an infant was determined and compared to the World Health Organization (WHO) recommendations and the Dietary Reference Intakes (DRI).

The study provides information regarding the composition of available CF and ways in which to improve them. It was anticipated that these three new CF would be more nutritious than those previously studied and that the analyses will allow for further re-modification of the formulas.

2. Materials and methods

2.1. CF

The three CF analyzed for this study were purchased at the Jos University Teaching Hospital (JUTH) and are used widely in northern Nigeria, particularly in the area of the Jos Plateau. The foods were developed in 2000 by a nutritionist, Margaret Williams, at JUTH. The CF were air-dried to constant weight and packaged using cereals and other components purchased in local markets. The ingredients were foodstuffs that are commonly used and abundantly available in the region. Ambient drying under conditions that prevail in northern Nigeria is sufficient to stabilize vis-à-vis microbial degradation. The abbreviations used for the foods were derived from the list of main ingredients for each food: the food composed of aya, tamba and carrots is abbreviated ATC, whereas the food containing acha, bambara nut, and rice is shortened to ABR and the food with the ingredients of maize, millet, sorghum and groundnuts is called MMSG (Tables 1 and 2).

These CF are normally prepared for consumption by adding 100 mL of boiling water per 10 g of food mix, resulting in a 9% (w/w) mixture. Preparation of the CF with boiling water discourages microbial growth and parasitic infestation. In addition to consuming human milk, it is estimated that a healthy 6–7-month-old-infant in Jos usually consumes approximately 65 g (dry weight) of CF per day. Prior to analysis, all CF were dried to constant weight under a vacuum (Alcatel, USA) at 25 °C.

2.2. Chemical analysis of CF

2.2.1. Fatty acid analysis

The dried powdered specimens were extracted with chloroform:methanol (2:1, v/v) as described elsewhere (Kim et al., 1997) and the solid, non-lipid material was removed by filtration. The total

Table 1
Samples from Nigeria: May 9th, 2000

Common name		Latin Name
Acha	(hungry rice)	<i>Digitaria exilis</i>
Aya	(yellow nut sedge)	<i>Cyperus escutanti</i>
Bambara Nut	(wild rice)	<i>Vigna subterranea</i>
Dawa	(red sorghum)	<i>Sorghum bicolor (aka guinea-corn)</i>
Doro	(millet) (maybe teff)	<i>Eragrostis tef or E. abyssinica</i>
Groundnuts	(peanuts)	<i>Arachis hypogaea</i>
Kuka	(baobab leaf)	<i>Adansonia digitata</i>
Maize	(yellow corn)	<i>Zea mays</i>
Rice	(white rice)	<i>Oryza</i>
Soyabean	(dehulled)	<i>Glycine max</i>
Tamba	(pwana)	<i>Eleusine coracana</i>
New weaning foods are described primarily by content.		
Portions, A, B and C have been assigned the numbers 1, 2 and 3.		
New weaning food #1	RABCC (g)	Portion A is in five 250 ml Nalgene bottles
Content		% composition
Rice	200	31
Acha	200	31
Bambara nut	200	31
Carrots	40	6
Crayfish	5	1
Total	645	100
New weaning food #2	New WF (g)	Portion 2 is in four 250 ml nalgene bottles
Content		% composition
Millet	125	21
Sorghum	125	21
Maize	125	21
Groundnuts	125	21
Soyabeans	50	8
Kuka	50	8
Crayfish	5	1
Total	605	100
New weaning food #3	ATC (g)	Anti-diarrhea formula
Content		% composition
Aya	200	24
Tamba	500	59
Carrot	150	18
Total	850	100

All samples 100 g unless otherwise noted.

Table 2
Description of certain samples

Aya	Small (7–1.1 cm) irregular shaped, ribbed, wrinkled, brown seed. Hard exterior with soft pulp-like interior.
Doro (Millet)	Smooth seed in a teardrop shape. 2 mm × 3 mm. White-ish/tan color.
Tamba (Pwana)	Roundish, dark red/brown seeds. 1–½ mm in diameter. Hard white interior.
Dawa (red sorghum)	Teardrop shaped, smooth seed. 4 mm × 5 mm. Exterior color was reddish tan to tan. Color of interior was white. Interior and exterior were hard.
Rice	White, fairly translucent, 2 mm × 5–7 mm.
Acha (<i>Digitaria exilis</i>)	Small (1 mm or smaller), slightly oval-shaped, hard seeds of a tan/yellow color. (aka Hungry rice).
Bambara nut (<i>Vigna subterranea</i>)	Large (1.1–1.3 cm), tan, hard seeds, slightly oval-shaped. Thin (⅓ mm) hard shell surrounding a tan hard interior. Interior is a shade lighter tan than the exterior.

extracted lipid material was recovered after solvent removal in a stream of nitrogen. The samples were then redissolved in chloroform:methanol (2:1, v/v) and centrifuged (International Equipment Company, USA). Transmethylation was performed using 14% (w/v) BF₃ in methanol (Morrison and Smith, 1964). Fifty nanograms of heptadecanoic acid (internal standard) and a 1 mL aliquot of each sample were transferred to a screw-cap tube. After removal of solvent, the sample was mixed with 2 mL of BF₃ reagent, placed in a warm bath (Thermolyne, USA) at 100 °C for 30 min and cooled. After the addition of 2 mL of saline solution, the transmethylated fatty acids were extracted into hexane.

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). One to two microliter aliquots of the hexane phase were injected in split-mode onto a fused-silica capillary column (Omegawax; 30 m × 0.32 mm ID, Supelco, Bellefonte, PA). The injector temperature was set at 200 °C, detector at 230 °C, oven at 120 °C initially, then 120–205 °C at 4 °C per min, 205 °C for 18 min. The carrier gas was helium and the constant flow rate was approximate 50 cm/s. The internal standard (heptadecanoic acid, 17:0) and calibration standards GLC-85 (Nu-chek, Elysian, MN) were used for quantitation of fatty acids in the various lipid extracts. Solvents were purchased from EM Science, Gibbstown, NJ. The data reported represent the average of three determinations from 1–2 samples of each CF. The coefficient of variance was less than or equal to 3%.

2.2.2. Amino acid analysis

Two to three milligrams of each CF were transferred, weighed and analyzed in a tared glass ampoule. The internal standard used in all determinations was 40 mmols of norleucine. After 1.0 mL of 6 N HCl was added, the samples were flushed with nitrogen, evacuated, sealed and

placed in an oven (Blue M Electric Co., USA) at 110 °C for 24 h. Following hydrolysis, a 10 µL aliquot was withdrawn and subjected to derivatization.

Samples to be used for the determination of cysteine were first oxidized with performic acid [formic acid: 30% hydrogen peroxide, 9:1 (v:v)] for 18 h at room temperature (Hirs, 1967). Performic acid was removed in an evaporative centrifuge (Speed-Vac, Savant) and the samples were hydrolyzed with HCl as described above.

The tryptophan content was determined separately. With regard to the tryptophan analysis, 450 µL of 4.67 M KOH containing 1% (w/v) thioglycol were added to each sample. Hydrolysis was performed within an evacuated ampoule at 110 °C for 24 h. After allowing the hydrolysate to cool, 0.5 mL of 4.2 M perchloric acid (PCA) and 50 µL of acetic acid were added to neutralize the solution. The samples were mixed thoroughly using a Thermolyne Maxi mixer, in preparation for derivatization. Duplicate lysozyme controls were analyzed for quality control purposes.

The samples were dried using 20 µL of ethanol:triethylamine:water (2:1:2, v/v) and derivatized with 20 µL of ethanol:triethylamine:water: phenylisothiocyanate (7:1:1:1, v/v). Excess reagent was removed in a speedvac. Derivatized and dried samples were dissolved in 100 µL of equilibration buffer.

Analysis of the amino acids was performed with a Waters C18 column (3.9 × 150 mm²). The gradient solution was the same as that described by Bidlingmeyer, Cohen, and Tarvin (1984). The solvents utilized were the sodium acetate buffer and acetonitrile (300 mL ACN, 200 mL water, 0.2 mL CaEDTA). Twenty microliter aliquots were injected onto the column. Tryptophan analysis was performed according to Hariharan, Naga, and VanNoord (1993). Amino acid derivatives were detected at 254 nm. The data reported represent the average of two determinations from 1–2 samples of each CF. The coefficient of variance was less than or equal to 4%.

2.2.3. Minerals

The samples were dried (Lab-Line Instruments, USA) overnight at 110 °C, then stirred and allowed to cool to room temperature. Three replicate aliquots containing approximately 0.1 g of each sample were weighed and then digested using 20 mL concentrated nitric acid and 1 mL concentrated perchloric acid. The samples were set on a hot-plate at 120 °C for 1 h, then at 150 °C to reflux overnight. The samples were then treated with 2.5 mL of nitric-perchloric acid (4:1, v/v), and a minimal amount of deionized water. After cooling, the solutions were diluted to 50 mL final volume with deionized water. The samples were analyzed by ICP-AES (Spectro Analytical Instruments, USA) for trace metals content. The instrument was calibrated using known standards for each mineral provided by the National Institute of Standards and Technology. The accuracy of the method was validated using tomato leaf standard of known mineral content. The digestion technique makes no attempt to solubilize any silicate-based materials that may be in the samples. The data reported represent the average of three determinations from 1 to 2 samples of each CF.

3. Results

Table 3 lists the ingredients of each of the three second-generation CF and compares them to the most nutritious first-generation CF (Soy). Because the second-generation CF are composed of

Table 3

Composition (%wt/wt) of three second-generation CF compared to a first-generation CF

Common name ^a	Local (Hausa) name	Latin name	Second-generation CF			First-generation CF
			ABR%	MMSG%	ATC%	Soy(%)
Hungry rice	Acha	<i>Digitaria exilis</i>	31	—	—	—
Yellow nut sedge	Aya	<i>Cyperus esculanti</i>	—	—	24%	—
Bambara nut	Bambara Nut	<i>Vigna subterranea</i>	31	—	—	—
Carrots	Carrots	<i>Daucus carota</i>	6	—	17%	—
Corn	Maize	<i>Zea mays</i>	—	21	—	—
Crayfish	Crayfish	<i>Procambarus ssp.</i>	1	1	—	—
Peanuts	Groundnuts	<i>Arachis hypogaea</i>	—	21	—	—
Baobab leaf	Kuka	<i>Adansonia digitata</i>	—	8	—	—
Rice	Shinkafa	<i>Oryza</i>	31	—	—	—
Sorghum	Dawa	<i>Sorghum bicolor</i>	—	21	—	—
Soy bean	Soyabean	<i>Glycine max</i>	—	8	—	100
Millet	Tamba	<i>Eleusine coracana</i>	—	20	59%	—

^aAll ingredients were air-dried to constant weight and ground to powder.

some ingredients previously studied (corn, millet, rice soy and others) but also include new ingredients that have not been analyzed (hungry rice, yellow nut sedge, bambara nut, baobab leaf, sorghum and millet) it was difficult to predict how the second-generation CF would compare to the first-generation CF in terms of nutrient quality. We anticipated, based on the nutrient content of the additional ingredients, that the second-generation CF would be more nutritious than the first-generation CF.

In order to identify the most nutritious CF for Nigerian infants, the nutrient contents of the three second-generation CF (ABR, MMSG and ATC) were compared to the most nutritious first-generation CF (Soy). A comparison of the fatty acid composition (mg/g) of the second-generation CF to the first-generation CF is provided in Table 4. All three new CF contained less saturated, monounsaturated, n-6 and n-3 fatty acids than Soy. When total daily nutrient intake was calculated assuming a child consumes 65 g (dry wt) of CF per day, all three second-generation CF contained much lower amounts of total fatty acids than Soy (0.25, 1.78 and 1.35 g/day, respectively, compared to 7.03 g/day).

In Table 5, the mineral content ($\mu\text{g/g}$) of the three second-generation CF is compared to that of the most nutritious first-generation CF. All three second-generation CF contained more aluminum, iron, lithium and nickel than Soy, but less potassium, magnesium, molybdenum, phosphorus and zinc.

Table 6 compares the amino acid content (mg/g) of the three second-generation CF to the most nutritious first-generation CF. Soy surpassed all three CF in total protein content (355 mg/g compared to 108, 197 and 66 mg/g) and total daily protein (23 g/65 g compared to 7.01, 12.8 and 4.28 g/65 g). Among the second-generation CF, MMSG contained more total protein than either ABR or ATC (12.8 g/65 g versus 7.01 and 4.28 g/65 g).

It is important to compare the second-generation CF to the first-generation CF in order to assess which offers better nutrition to infants in Nigeria. However, a more basic question must be

Table 4

Fatty acid composition (mg/g) of three second-generation CF compared to a first-generation CF

Fatty acid	Second-generation CF ^a						First-generation CF ^b
	ABR (mg/g) (s.d.)		MMSG (mg/g) (s.d.)		ATC (mg/g) (s.d.)		Soy (mg/g)
Saturated							
C14:0	ND		0.02	(0.00)	0.02	(0.00)	0.13
C16:0	0.66	(0.07)	3.04	(0.08)	3.21	(0.09)	13.3
C18:0	0.24	(0.02)	0.96	(0.04)	1.37	(0.03)	3.42
Total	1.23	(0.13)	5.78	(0.13)	5.11	(0.13)	16.9
Monounsaturated							
C16:1	0.01	(0.01)	0.04	(0.00)	0.05	(0.00)	0.16
C18:1 n-9	0.83	(0.08)	10.2	(0.44)	12.7	(0.29)	20.9
Total	0.87	(0.10)	10.6	(0.42)	12.8	(0.30)	21.1
n-6 Fatty acids							
C18:2 n-6	1.64	(0.16)	10.1	(0.28)	2.57	(0.05)	59.7
C20:4 n-6	ND		ND		ND		ND
Total	1.65	(0.16)	10.1	(0.27)	2.57	(0.05)	59.7
n-3 Fatty acids							
C18:3 n-3	0.08	(0.01)	0.63	(0.02)	0.15	(0.01)	7.46
C20:5 n-3	0.01	(0.00)	ND		ND		ND
C22:6 n-3	0.02	(0.00)	0.01	(0.01)	ND		ND
Total	0.11	(0.01)	0.64	(0.02)	0.15	(0.01)	7.46
Total fatty acid (mg/g)	3.89	(0.39)	27.4	(0.81)	20.8	(0.47)	105
Total fatty acid per daily intake (g/65 g)	0.25		1.78		1.35		7.03

ND—not detected.

s.d.—standard deviation.

^aData presented are the average of three determinations.^bData presented are the average of two determinations.

answered: Are the second-generation CF sufficiently nutritious when compared to World Health Organization (WHO) recommendations, dietary reference intakes (DRI) and recommended dietary allowances (RDA)?

Table 7 compares the fatty acid compositions of the three second-generation CF to WHO recommendations. The WHO values were constructed based on the recommendation that CF have a similar fatty acid composition to breast milk. Medians were calculated from average fatty acid values of human milk in Europe and Africa (Koletzko, Thiel, & Abiodun, 1992).

All three second-generation CF contained moderate total unsaturated fat and low total saturated fat, which resulted in high total unsaturated/saturated fatty acid ratio. The three CF also contained moderate to high proportions of α -linolenic acid (18:3n3) and high proportions of linoleic acid (18:2n6), which resulted in a high total n-6/total n-3 ratio and a high linoleic/ α -linolenic acid ratio.

Assuming a daily intake of 65 g of dry CF per day, the mineral content of the three second-generation CF were compared to the Dietary Reference Intakes (Table 8). The (DRI) are a set of four reference values which include the RDA, estimated average requirement (EAR), adequate

Table 5

Mineral content ($\mu\text{g/g}$) of three second-generation CF compared to a first-generation CF

Mineral	Second-generation CF ^a						First-generation CF ^b
	ABR		MMSG		ATC		Soy
	$\mu\text{g/g}$	(S.D.)	$\mu\text{g/g}$	(S.D.)	$\mu\text{g/g}$	(S.D.)	$\mu\text{g/g}$
Ag	0.39	(0.10)	0.40	(0.05)	0.45	(0.09)	<0.85
Al	73.1	(25.3)	216	(17.42)	72.9	(6.84)	26.8
As	2.98	(0.23)	3.69	(0.35)	3.13	(0.14)	<6.00
Ba	5.55	(0.04)	26.6	(0.68)	39.6	(0.31)	18.9
Be	0.04	(0.01)	0.06	(0.00)	0.05	(0.00)	<0.068
Ca	1384	(165.76)	2720	(176.32)	2662	(77.24)	2685
Cd	<0.155		<0.155		0.22	(0.05)	<0.36
Co	0.32	(0.03)	0.36	(0.01)	0.41	(0.01)	<0.41
Cr	0.65	(0.05)	1.05	(0.22)	0.77	(0.09)	0.72
Cu	6.93	(0.67)	12.6	(4.81)	6.82	(0.07)	12.4
Fe	140	(26.66)	288	(56.37)	138	(4.76)	98.1
K	5354	(164.43)	5706	(133.67)	9078	(277.10)	11.600
La	0.45	(0.11)	0.73	(0.06)	0.83	(0.03)	<1.50
Li	0.73	(0.09)	0.82	(0.02)	0.69	(0.04)	0.06
Mg	1030	(38.76)	1733	(1.53)	1428	(8.02)	2255
Mn	10.2	(0.52)	21.8	(1.46)	254	(16.99)	34.2
Mo	1.78	(0.04)	1.97	(0.06)	1.11	(0.07)	5.71
Na	991	(78.51)	428	(13.40)	1585	(84.72)	<650
Ni	1.14	(0.10)	2.72	(0.04)	1.65	(0.08)	0.91
P	2237	(52.16)	3830	(72.00)	2734	(13.01)	5685
Pb	2.29	(0.25)	2.73	(0.05)	2.81	(0.11)	<2.40
Sb	4.43	(1.49)	5.73	(1.17)	5.79	(1.91)	<5.90
Se	4.14	(0.12)	5.34	(0.37)	4.59	(0.23)	<4.60
Sr	14.8	(2.06)	25.4	(1.39)	14.8	(0.02)	15.6
Te	2.52	(0.17)	2.93	(0.15)	3.30	(0.74)	<8.70
Ti	1.12	(0.61)	1.71	(0.19)	1.10	(0.08)	<0.58
Tl	1.75	(0.02)	2.42	(0.18)	2.45	(0.14)	2.24
V	0.86	(0.07)	1.14	(0.01)	0.93	(0.01)	<0.92
Y	0.07	(0.02)	0.20	(0.01)	0.18	(0.01)	<0.25
Zn	28.9	(3.30)	37.9	(1.54)	33.6	(1.37)	54.8
Zr	0.47	(0.13)	0.65	(0.07)	0.51	(0.08)	<1.80

S.D.—standard deviation.

^aData presented are the average of three determinations.^bData presented are the average of two determinations.

intake (AI), and tolerable upper intake level (UL). The RDA is the average daily dietary intake level that is sufficient to meet the nutrient requirement of nearly all (97–98%) apparently healthy people. The EAR is the dietary intake level that is estimated to meet the nutritional needs of fifty percent of healthy people in an age- and gender-specific group. AI is a recommended average daily nutrient intake level that is assumed to be adequate. AI is based on experimentally derived intake levels or approximations of observed nutrient intake by a group (or groups) of apparently healthy

Table 6

Amino acid content (mg/g) of three second-generation CF compared to a first-generation CF

Amino acid	Second-generation CF ^a			First-generation CF ^b
	ABR	MMSG	ATC	Soy
Ala	7.03	8.12	3.42	13.0
Arg	6.26	14.0	4.59	25.4
Asp	8.05	14.2	4.36	36.2
Cys	1.73	9.98	0.97	4.37
Glu	18.9	35.0	14.7	63.5
Gly	3.88	8.81	2.61	12.9
His	3.07	4.73	1.89	9.44
Ile	4.44	7.98	2.86	16.9
Leu	8.98	16.3	5.46	26.4
Lys	5.39	8.35	2.32	21.6
Met	4.99	16.4	2.78	5.18
Phe	5.97	10.3	3.24	20.7
Pro	8.47	12.0	4.49	18.1
Ser	5.62	8.31	2.74	18.8
Thr	1.65	5.60	2.08	13.0
Trp	2.06	2.36	1.45	19.1
Tyr	3.32	4.92	1.98	12.4
Val	8.00	9.35	3.91	17.8
Total (mg/g)	108	197	65.9	355
Total daily protein (g/65 g)	7.01	12.8	4.28	23.1

^aData presented are the average of three determinations.^bData presented are the average of two determinations.

Table 7

Fatty acid composition (%wt/wt) of three second-generation CFs compared to WHO recommendations

Fatty acid	Second-generation CF ^a			Recommended ^b
	ABR	MMSG	ATC	
Linoleic acid 18:2n-6	42.2	37.0	12.4	11.0–12.0
α -Linolenic acid 18:3n-3	2.16	2.30	0.71	0.8–0.9
Arachidonic acid 20:4n-6	0.12	0.00	0.00	0.5–0.6
Docosahexaenoic acid 22:6n-3	0.46	0.02	0.00	0.3–0.3
Total unsaturated	44.9	39.3	13.1	44.8–52.4
Total saturated	31.6	21.1	24.6	45.2–53.5
Unsaturated/saturated ratio	2:1	4:1	3:1	0.84:1–1.16:1
18:2n-6/18:3n-3 ratio	20:1	16:1	17:1	5:1–10:1
Total n-6/total n-3 ratio	15:1	16:1	17:1	2.4:1–2.7:1

NR,—not reported.

^aData presented are the average of three determinations.^bRecommended data from Koletzko et al., 1992. Medians calculated from average FA values reported of human milk in Europe and Africa. Ratio of 18:2 to 18:3 as suggested by the FAO/WHO Joint Consultation, 1995.

Table 8

Daily consumption of three second-generation CF compared to dietary reference intakes

Mineral	(Units)	Second-generation CF ^a			Dietary reference intakes ^b	
		ABR	MMSG	ATC	Adequate intakes	Upper limits
As	(µg/day)	194	240	204	NA	NA
Ca	(mg/day)	90	177	173	270	NA
Cr	(µg/day)	42.4	68.0	50.2	5.50	NA
Cu	(µg/day)	450	822	444	220	NA
Fe	(mg/day)	9.10	18.7	8.98	11.0 ^c	40.0
Mg	(mg/day)	66.9	113	92.8	75.0	NA
Mn	(mg/day)	0.66	1.41	16.5	0.60	NA
Mo	(µg/day)	115	128	72.2	3.00	NA
Ni	(mg/day)	0.07	0.18	0.11	NA	NA
P	(mg/day)	145	249	178	275	NA
Se	(µg/day)	269	347	298	20.0	60.0
V	(mg/day)	0.06	0.07	0.06	NA	NA
Zn	(mg/day)	1.88	2.46	2.18	3.00 ^d	5.00

NA—not available.

^aData presented are the average of three determinations.^bDietary Reference Intakes for infants 7–12 months. From the Food and Nutrition Board, sources: 1997, 1998, 2000, 2001, 2002. The adequate intake for healthy breastfed infants is the mean intake. Upper limits are the maximum level of a daily nutrient intake that is likely to pose no risk of adverse effects.^cRDA, assuming a moderate bioavailability of 10% iron absorption.^dRDA.

people. UL is the highest level of daily nutrient intake that is likely to pose no risk of adverse health effects (Institute of Medicine, US, 2000).

All three second-generation CF were found to provide more than adequate intake levels for chromium, copper, manganese and molybdenum (Institute of Medicine, US, Panel on Micronutrients, 2002a). The selenium content of each of the second-generation CF was above the upper limit of the DRI. ABR, MMSG and ATC contained 269, 347 and 298 µg of selenium per day, respectively, compared to the recommended upper limit of 60 µg per day (Institute of Medicine, US, 2000).

However, all three second-generation CF were found to contain relatively low amounts of calcium, phosphorus and zinc. MMSG contained the highest levels of most essential minerals, with the exception of manganese (Institute of Medicine, US, 1997). Only MMSG met the RDA for iron, assuming a medium bioavailability of 10%.

In order to estimate the total amount of amino acid provided per day by the second-generation CFs, the total protein content per CF (mg/g) was multiplied by 65 g (Table 6). When compared to the RDA of protein for a 7–12-month-old infant (13.5 g/day), all three CF were found to be inadequate (ABR, MMSG and ATC were 7.01, 12.8 and 4.28, respectively) (Institute of Medicine, US, Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, 2002). However, MMSG provided the most protein of the second-generation CF.

Table 9 compares the amino acid content (mg/day) of the three second-generation CF to the RDA for infants 7–12-months-old (Institute of Medicine, US, Standing Committee on the

Table 9

Predicted amino acid composition (mg/day) of three second-generation CF compared to the RDA

Essential amino acid	RDA for infants 7–12 month old ^a	Second-generation CF		
		ABR ^b	MMSG ^b	ATC ^b
His + Arg	288	200	307	123
Ile	387	289	519	186
Leu	837	584	1061	355
Lys	801	350	543	151
Met + Cys	387	437	1712	244
Phe + Tyr	756	604	987	339
Thr	441	107	364	135
Trp	117	134	153	94
Val	522	520	608	254

^aRDA for the total daily intake of an infant weighing 9 kg. National Academies Press, 2002.

^bData presented are the average of two determinations.

Scientific Evaluation of Dietary Reference Intakes, 2002b). All three CF were deficient in lysine and threonine. Only MMSG would provide adequate amounts of the other essential amino acids. ATC did not have any essential amino acids that met the RDA.

4. Discussion

The results of the present study establish soy-based CF as superior in overall nutritive value to three available Nigerian CF that are based on rice, corn or millet. Superior overall nutritive value is defined as containing greater amounts of fatty acids, protein and minerals. In a previous study (Fernandez et al., 2002) the authors analyzed five first-generation CF and found that a soy-based formula (Soy) had the highest amount of essential fatty acids and protein, although it did not meet the RDA of calcium and zinc. The three second-generation CF (ABR, MMSG, and ATC) were developed with the goal of maximizing the nutritional content of locally produced Nigerian CF. The second-generation mixtures were based on rice, corn, millet, and other local foodstuffs. Only one, MMSG, contained soy beans (8%). Although the Soy CF was found to have a higher total protein and fatty acid content than all three second-generation CF and higher levels of phosphorus, magnesium, zinc and (with the exception of MMSG) calcium, the question remained: Could the three second-generation CF still meet basic dietary recommendations? If used properly, were they adequate sources of nutrition?

In analyzing the nutritional value of the second-generation CF, we made several assumptions regarding their use: first, that the recommendations of the 47th World Health Assembly for the introduction of CF were followed, and second, that children would begin to wean off breast milk at 6 months of age (47th World Health Assembly, 1994). Introducing CF prior to this age poses health risks for the infant. Microbial contamination of the CF can lead to infectious diarrhea, dehydration and severe illness, while cessation of breastfeeding results in a significant reduction in energy intake. If exclusive breastfeeding continues past 6 months of age, the nutrient needs of a growing infant cannot be met by breast milk alone.

A third assumption was that CF would be used in conjunction with breastfeeding to gradually introduce the infant to an adult diet. When CF are used in weaning, the child receives nutrition from both breast milk and CF. Some nutrients, such as fatty acids, are abundant in breast milk. However, low concentrations of other nutrients in breast milk, such as zinc and iron, may lead to clinically significant deficiencies if supplementation with CF is not provided (Dewey, 2001). Therefore, in assessing the overall nutritive intake of infants, the combined composition of breast milk and CF needs to be taken into consideration.

Current dietary recommendations suggest that fat should comprise 30–45% of the daily energy intake for children under two years of age. Brown et al. (1998) have concluded that infants age 6–8 months who are consuming an average amount of breast milk per day (674 g/day) do not need dietary supplementation of fatty acids in order to achieve 30% of the daily energy intake. If the breast milk has a low fat concentration, as may be the case in developing countries, infants require an additional 10–24% of energy from fat.

By the time infants reach 9 months of age, their CF diet should provide 13% of the energy from fat. The three second-generation CF fall below the amount needed (1.06–7.01% energy from fat). However, if maize or soybean oil were added in the preparation of CF, the fat content would be satisfactory. Maize and soybean oil, which contain large amounts of linoleic acid and α -linolenic acid, would also provide adequate amounts of these two essential fatty acids.

The essential fatty acids (EFA), α -linolenic acid and linoleic acid are precursors to docosahexaenoic acid and arachidonic acid that are critical for growth and development, particularly of the nervous system in the first 6 months of life. Both are necessary components of myelin, synaptic cell membranes and photoreceptor cells. The WHO report on complementary feeding concludes that diets low in fat may be adequate, as long as the minimum requirements for EFA are met (Brown et al., 1998). The three second-generation CF meet or exceed the WHO recommendations. However, the linoleic acid to α -linolenic acid ratio is higher than advised. The concern is that the relatively high levels of linoleic acid might inhibit the synthesis of docosahexaenoic acid from α -linolenic acid (Ballabriga, 1994).

According to WHO, the daily intake of protein needed from CF is 2.0–3.1 g per day for babies from 6 to 11 eleven months of age and who have an average breast milk intake. The second-generation CF exceeded the recommendations by a factor of two to six times (4.3–12.8 g/day). Although all three CF had lower total protein content than first-generation Soy, all of them do provide adequate levels of protein for a child who consumes an average amount of breast milk.

Some studies suggest that excessive protein intake may compromise an infant's long-term health. Although controversial, infant formulas in industrialized countries, which are high in protein, have been associated with obesity later in life (Rolland-Cachera et al., 1999). The current debate on infant protein dietary intake highlights the need to develop CF that have adequate, but not excessive protein content.

CF are important sources of micronutrients for the developing infant. Past the age of 6 months breast milk becomes insufficient to meet the needs of the growing child. Iron, zinc, phosphorus, magnesium, and calcium have been identified as deficient nutrients and must be supplemented by the addition of CF (Brown et al., 1998). The three second-generation CF were found to be incapable of meeting most of the mineral requirements of infants, as defined by WHO for infants consuming an average amount of breast milk. Calcium and zinc intakes were below the daily requirements for infants from ages 6 to 23 months. Only one of the CF, namely MMSG,

contained enough calcium and zinc to meet the needs of children from 12 to 23 months of age. However, all three CF contained adequate amounts of magnesium to meet the needs of children from age 6 to 23 months.

The absorption and utilization of iron in CF, which contain cereals and legumes, is significantly lower than from meat, fish or poultry. The bioavailability of non-heme iron found in plant sources is 2–8%, compared to 25% bioavailability of heme iron found in animal products. (Brown et al., 1998) Phytic acid, soybean protein and tannic acid have all been shown to inhibit the absorption of iron (Institute of Medicine, Panel on Micronutrients, 2002). Ascorbic acid reduces the effect of phytates and enhances the absorption of non-heme iron. The RDA for infants aged 7 to 12 months is based on the assumption that CF diets contain little meat and are high in cereals and vegetables. The bioavailability of iron in such diets is classified as medium and estimated to be 10%. Very restricted vegetarian diets have a low bioavailability of 5%. Only MMSG was found to meet the RDA for iron.

A limitation of the present study is the lack of analysis of the phytate content of the CF. Phytates affect the absorption of zinc and calcium, in addition to iron. They form complexes with minerals making them insoluble and inhibit their absorption. A quantification of the phytate content would provide a more accurate assessment of the mineral nutritive value provided by the CF. Soaking (Hotz, Gibson, & Temple, 2001), germination (Sanni, Asiedu, & Ayernor, 2001, Ibrahim, Habiba, Shatta, & Embaby, 2002) and fermentation (Sanni, Onilude, & Ibidapo, 1999, Makokha, Oniang'o, Njoroge, & Kamar, 2002) have been shown to reduce the phytate content of cereals and legumes and increase the bioavailability of minerals. Fermentation has also been shown to reduce the polyphenol and tannin contents of cereals and soybeans (Onilude et al., 1999).

Although the three CFs reported in this communication do not match the overall nutrient quality of Soy, they may have some other attributes that affect infant nutrition. For example, CF in northern Nigeria where the study was performed are used not just as a means of providing nutrients, but are also used to treat diarrheal disease. A study to compare the effectiveness of both the first- and second-generation CF in the management of diarrhea in infants is in progress.

In summary, although the three second-generation CF were less nutritious overall than the first-generation Soy product, they do provide adequate quantities of fat and protein (assuming infants receive an average amount of breast milk concurrently). However, the three CF contained insufficient concentrations of calcium, phosphorus, zinc and iron to meet the estimated daily needs of infants.

References

- Ballabriga, A., 1994. Essential fatty acids and human tissue composition. An overview. *Acta Paediatrica Scandinavica*, 402, 63–68.
- Bentley, M.E., Dickin, K.L., Mebrahtu, S., Kayode, B., Oni, G.A., Verzosa, C.C., Brown, K.H., Idowu, J.R., 1991. Development of a nutritionally adequate and culturally appropriate weaning food in Kwara State, Nigeria: an interdisciplinary approach. *Social Science & Medicine* 33, 1103–1112.
- Bidlingmeyer, B.A., Cohen, S.A., Tarvin, T.L., 1984. Rapid analysis of amino acids using pre-column derivatization. *Journal of Chromatography* 336, 93–104.

- Brown, H.H., Dewey, K.G., Allen, L.H., 1998. Complementary feeding of young children in developing countries: a review of current scientific knowledge. World Health Organization, Geneva, WHO/NUT/98.
- Dewey, K.G., 2001. The evidence for breastfeeding: nutrition, growth and complementary feeding of the breastfed infant. *Pediatric Clinics of North America* 48, 87–104.
- Fernandez, D.R., Vanderjagt, D.J., Williams, M., Okolo, S.N., Huang, V., Chuang, L., Glew, R.H., 2002. Fatty acid, amino acid and trace mineral analysis of five weaning foods from Jos, Nigeria. *Plant Foods for Human Nutrition* 57, 257–274.
- Guptill, K.S., Esrey, S.A., Oni, G.A., Brown, K.H., 1993. Evaluation of a face-to-face weaning food intervention in Kwara State, Nigeria: Knowledge, trial, and adoption of a home-prepared weaning food. *Social Science & Medicine* 36, 665–672.
- Hariharan, M., Naga, S., VanNoord, T., 1993. Systematic approach to the development of plasma amino acid analysis by high-performance liquid chromatography with ultraviolet detection with precolumn derivatization using phenyl isothiocyanate. *Journal of Chromatography* 621, 15–22.
- Hautvast, J.L.A., van der Heijden, L.J.M., Luneta, A.K., van Staveren, W.A., Tolboom, J.J.M., van Gastel, S.M., 1999. Food consumption of young stunted and non-stunted children in rural Zambia. *European Journal of Clinical Nutrition* 53, 50–59.
- Hirs, C.W.H., 1967. Performic acid oxidation. *Methods Enzymology* 11, 197–199.
- Hotz, C., Gibson, R.S., Temple, L., 2001. A home-based method to reduce phytate content and increase zinc bioavailability in maize-based complementary diets. *International Journal of Food Sciences and Nutrition* 52, 133–142.
- Ibrahim, S.S., Habiba, R.A., Shatta, A.A., Embaby, H.E., 2002. Effect of soaking, germination, cooking and fermentation on antinutritional factors in cowpeas. *Nahrung* 46, 92–95.
- Institute of Medicine, US, 1997. Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride. National Academy Press, Washington, DC.
- Institute of Medicine, US, 2000. Panel on Dietary Antioxidants and Related Compounds, DRI, Dietary reference intakes: for vitamin C, vitamin E, selenium, and carotenoids: a report of the Panel on Dietary Antioxidants and Related Compounds, Subcommittees on Upper Reference Levels of Nutrients and Interpretation and Uses of DRIs, and the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, Institute of Medicine. National Academy Press, Washington, DC.
- Institute of Medicine, US, Panel on Micronutrients, 2002a. DRI: dietary reference intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. A Report of the Panel on Micronutrients, Subcommittees on Upper Reference Levels of Nutrients and of Interpretation and Use of Dietary Reference Intakes, and the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, Institute of Medicine. National Academy Press, Washington, DC.
- Institute of Medicine, US, Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, 2002b. Dietary Reference Intakes for Energy, Carbohydrates, Fiber, Fat, Protein and Amino Acids (Macronutrients). A Report of the Panel on Macronutrients, Panel on the Definition of Dietary Fiber, Subcommittees on Upper Reference Levels of Nutrients and Interpretation and Uses of Dietary Reference Intakes, and the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. The National Academies Press, Washington, DC.
- Jansen, G.R., 1992. Centrally processed weaning foods for use in developing countries. *Food Reviews International* 8, 307–346.
- Kim, T.S., Pastuszyn, A., VanderJagt, D.J., Glew, R.S., Milson, M., Glew, R.H., 1997. The Nutritional composition of seeds from *Boscia senegalensis* (Dilo) from the Republic of Niger. *Journal of Food Composition Analysis* 10, 73–81.
- Kleinman, R.E., 1998. *Pediatric Nutrition Handbook*, fourth ed. American Academy of Pediatrics, Elk Grove Village, IL.
- Koletzko, B., Thiel, I., Abiodun, P.O., 1992. The fatty acid composition of human milk in Europe and Africa. *Journal of Pediatrics* 120, S62–S70.

- Makokha, A.O., Oniang'o, R.K., Njoroge, S.M., Kamar, O.K., 2002. Effect of traditional fermentation and malting on phytic acid and mineral availability from sorghum (*Sorghum bicolor*) and finger millet (*Eleusine coracana*) grain varieties grown in Kenya. *Food and Nutrition Bulletin* 23 (Suppl3.), 241–245.
- Morrison, W.R., Smith, L.M., 1964. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron trifluoride methanol. *Journal of Lipid Research* 5, 600–608.
- Nout, M.J.R., 1993. Processed weaning foods for tropical climates. *International Journal of Food Science Nutrition* 43, 213–221.
- Onilude, A.A., Sanni, A.I., Ighalo, M.I., 1999. Effect of process improvement on the physico-chemical properties of infant weaning food from fermented composite blends of cereal and soybeans. *Plant Foods for Human Nutrition* 43, 239–250.
- Rolland-Cachera, M.F., Deheeger, M., Bellisle, F., 1999. Increasing prevalence of obesity among 18-year-old males in Sweden: evidence for early determinants. *Acta Paediatrica* 88, 365–367.
- Sanni, A.I., Asiedu, M., Ayernor, G.S., 2001. Influence of processing conditions on the nutritive value of Ogi-baba, a Nigerian fermented sorghum gruel. *Plant Foods for Human Nutrition* 56, 217–223.
- Sanni, A.I., Onilude, A.A., Ibadapo, O.T., 1999. Biochemical composition of infant weaning food fabricated from fermented blends of cereal and soybean. *Food Chemistry* 65, 35–39.
- Werk, L.N., Alpert, J.J., 1998. Solid feeding guidelines. *Lancet* 352 (9140), 1569.
- 47th World Health Assembly, 1994. Infant and young child nutrition, resolution WHA47.5.
- World Health Organization/Food and Agriculture Organization, 1995. WHO and FAO joint consultation: fats and oils in human nutrition. *Nutrition Review* 53, 202–205.