



Analysis of the Nutrient Content of Infant Complementary Food Fortificant-*Moringa oleifera* Leaves with the Commonly Consumed Local Infants Foods in Nigeria: *Zea mays* and *Glycine max*

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJTDH/2014/11949

Editor(s):

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(3) Anonymous, Maize Research Institute, Serbia.

Peer review History: <http://www.sciencedomain.org/review-history.php?iid=624&id=19&aid=5825>

Short Communication

**Received 11th June 2014
Accepted 1st August 2014
Published 21st August 2014**

ABSTRACT

Aims: Analysis of the nutrient content of maize (*Zea mays*), soybean (*Glycine max*) and *Moringa oleifera* leaves commonly consumed by infants in Nigeria were done to determine the nutrient composition of the complementary foods and leaves.

Methodology: Yellow maize (*Zea mays*) grain were fermented for 48h and oven-dried. Soybean (*Glycine max*) seeds were boiled for 1h, dehulled and oven-dried. *Moringa oleifera* leaves were shade-dried. All the food materials were milled into fine flours. The proximate, energy, mineral and β -carotene contents of the flours were determined using standard methods. The result was analyze using SPSS version 17 to determine the standard deviation (SD) and percentage nutrient composition in 100g sample.

Results: The proximate and energy composition of food materials (Maize, soybean and *Moringa oleifera* leaves) used. The crude protein level was 36.46% in soybean, 27.45% and 10.22% in *Moringa oleifera* leaves and maize respectively. The energy level was 1,849KJ in soybean, 1,531KJ in maize and 1,134KJ in *Moringa oleifera* leaves. The minerals and β -carotene contents of maize, soybean and *Moringa oleifera* leaves (%). Calcium was 1,335mg in *Moringa oleifera* leaves, 14.49mg in soybean and 11.67mg in maize. *Moringa oleifera* leaves had 26.44mg iron, soybean had 9.65mg and maize had 5.95mg. Zinc level was 7.49mg in *Moringa oleifera* leaves, 3.46mg in soybean and 1.87mg in maize. β -carotene was 3,846.15RE in *Moringa oleifera* leaves, 538.46RE in soybean and 102.56RE in maize.

Conclusion: Incorporation of pulverized *Moringa oleifera* leaves in infants' food could diversity food intake, ensure food security and reduce some micronutrient deficiency diseases.

Keywords: Nutrient content; complementary food fortificant; *Moringa oleifera*, *Zea may* and *Glycine max*.

1. INTRODUCTION

Malnutrition is a major public problem of infants and young children in Nigeria. The problem is more common amongst children aged 6 months to two years. This coincides with the period of complementary feeding-when the family food is gradually being introduced to the child [1,2]. Research showed that protein energy malnutrition (PEM) at 6 months to 2 years was 43.1% while moderate and severe malnutrition was 22.3% in Nigeria [3]. There is equally high rate/level of stunting and wasting among these children. The high rate of malnutrition was attributed to the infants been introduced to poor complementary foods, which are inadequate in protein, energy and micronutrients. In effect, vitamin A deficiency among these children is higher than 16% in some regions in Nigeria [4] while anemia is as high as 50% [3].

Protein-energy malnutrition (PEM) or protein-calorie malnutrition refers to a form of malnutrition where there is inadequate calorie or protein intake.

Types include [5]:

- Kwashiorkor (protein malnutrition predominant)
- Marasmus (deficiency in calorie intake)
- Marasmic Kwashiorkor (marked protein deficiency and marked calorie insufficiency signs present, sometimes referred to as the most severe form of malnutrition)

Note that this may also be secondary to other conditions such as chronic renal disease [6] or cancer cachexia [7] in which protein energy wasting may occur. Protein–energy malnutrition affects children the most because they have less protein intake. The few rare cases found in the developed world are almost entirely found in small children as a result of fad diets, or ignorance of the nutritional needs of children, particularly in cases of milk allergy [8].

Infants and young children are one of the vulnerable groups in the society. They are more vulnerable when they are denied their rights to proper feeding and good nutrition. Nigeria ranks 14th in global assessment of under-five mortality rate and approximately one million children die annually in Nigeria before their 5th birthday [9]. Nigeria is among the 20 countries in the world that account for 80% of undernourished children. The causes of this public health problem in Nigeria are complex and multidisciplinary. However, poor quality and quantity of foods given to children play a major role [10].

WHO recommended the major criteria for a good-quality complementary food to be adequate protein content, high energy value per unit of food volume, soft texture, low fiber content, adequate vitamins and minerals and absence of anti-nutritional factors. To this effect, the formulation and development of nutritious complementary foods from local and readily available foods had received considerable attention in many developing countries [11]. However, the development of low cost, high protein and micronutrient dense food supplements for infants is a constant challenge for developing countries [12]. This is particularly important in counties like Nigeria where malnutrition is still common.

Unfortunately, a lot of works have been done which addressed protein-energy malnutrition, but there is still gap in micronutrient deficiency problem. To address these nutritional problems, nutritious complementary foods could be formulated from locally available foods. This study would explore the nutrient content of maize (*Zea mays*), soybean (*Glycine max*) and *Moringa oleifera* leaves commonly consumed by infants in Nigeria.

2. METHODOLOGY

2.1 Materials

Yellow maize (*Zea mays*) seeds, soybean (*Glycine max*) seeds and *Moringa oleifera* (Drum stick) leaves were used for the study. The maize and soybean were purchased in bulk from New Market, Enugu. The *Moringa oleifera* leaves were obtained from trees in a family farm in the Government Reserved Area (GRA) Enugu, Nigeria.

2.2 Preparation of Materials

2.2.1 Maize

Eighteen kilogrammes (18kg) of yellow maize were hand-picked and cracked with a laboratory harmer-mill (Thomas Willey, mold ED-5) to separate the bran from the grain. The cracked grains were winnowed to remove the bran. The seeds were then steeped in water in a ratio of 1:3 weight of grain/volume of water (w/v) and allowed to ferment at 28+2°C (ambient temperature) for 48h by the micro flora inherent in the seed. The water was changed after 24h to remove sour taste. At the end of the fermentation period, the grains were drained, dried in hot air oven at 55°C for about 10h, dry milled and packaged in

polythene bags as fermented maize flour and stored frozen at a temperature of -4°C until when used. Fig. 1 below describes the steps used to process the maize.

2.2.2 Soybean

Fifteen kilogrammes (15kg) of soybean seeds were cleaned by hand-picking. The samples were boiled with water in the ratio of 1:3 weight of seeds/volume of water (w/v) for 1h. The grains were dehulled manually and dried in a hot air oven (Gallenkamp BS oven, model 320) at 55°C for about 10h and milled in a laboratory harmer mill (Thomas Willy, Model ED-5) to a fine flour (70mm mesh screen). The flour was packaged in a polythene bag and stored frozen at -4°C until used. Fig. 2 below describes the steps used to process the soybean.

2.2.3 Moringa oleifera leaves

Eight kilogrammes (8kg) of fresh *Moringa oleifera* leaves were plucked, washed and drained, shade-dried, pulverized and packaged in polythene bag and stored in the freezer at -4°C until used. Fig. 3 below describes the steps used to process the *Moringa oleifera* leaves.

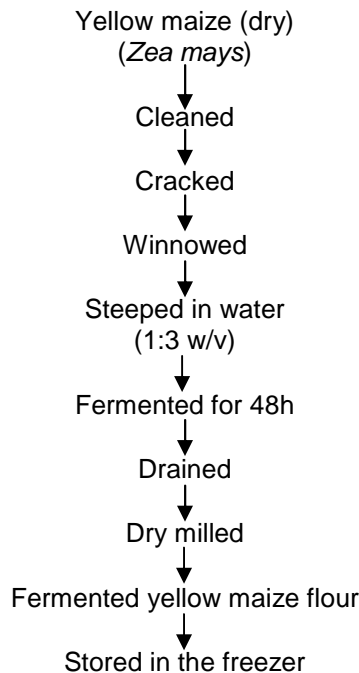


Fig. 1. Processing of maize

2.3 Chemical Analysis

The samples were subjected to chemical analysis in the laboratory to determine the proximate, mineral and vitamin composition. The various procedures used are described below.

2.4 Proximate Analysis

2.4.1 Determination of moisture content

Determination of moisture content was done using AOAC method [13,14]. About two grammes (2g) of sample were weighed into a preweighed clean weighing/drying dish provided with easily removable lid. The dish was uncovered and placed in a well-ventilated oven maintained at 103°C±2°C. After 16h, the lid was replaced and transferred to a desiccators at room temperature to cool, and quickly weighed to 0.1g and placed in the oven for another two h. Steps 4 and 5 were repeated until the mass was decreased between successive weighing not exceeding 0.06mg per g of sample (fresh weight basis). The loss in weight represented percent moisture content as follows:

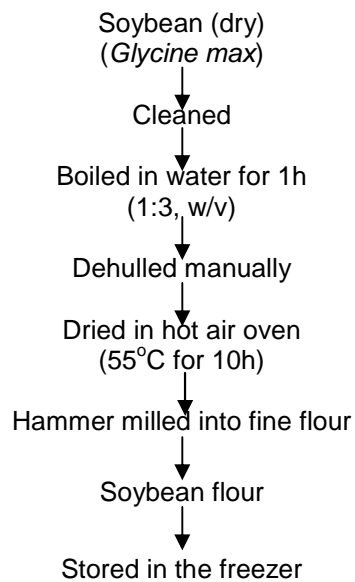


Fig. 2. Processing of soybean

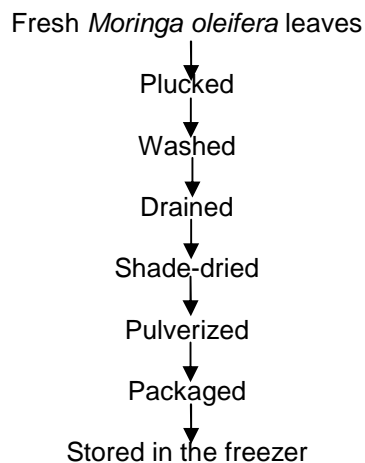


Fig. 3. Processing of *Moringa oleifera* leaves

$$\% \text{ moisture} = \frac{M_1 - M_2}{M_1 - M_0} \times 100$$

Where, M_0 = weight of dish and lid
 M_1 = weight in g of dish, lid and sample before drying
 M_2 = weight in g of dish, lid and sample after drying

Note that $M_1 - M_0$ = weight of sample prepared for drying

2.4.2 Determination of ash content (AOAC,)[13,14]

Crucible was washed and dried in an air hot oven, cooled in a dessicator and reweighed. About 2g of dried sample were weighed into the empty porcelain crucible previously ignited over a hot plate in the fume cupboard to char organic matter. The crucible was placed in a muffle furnace maintained at a temperature of 600°C for 6h, transferred to a dessicator, cooled and reweighed immediately.

$$\% \text{ Ash} = \frac{(\text{weight of crucible+ash}) - \text{wt of empty crucible}}{\text{Sample weight}} \times 100$$

2.4.3 Determination of crude fibre, Joselyn method [5,6]

About 2g of the sample were weighed into a 600ml long beaker. 200ml of hot 1.25% H_2SO_4 was added. Beaker was placed on digestion apparatus with preheated plates, boiled, refluxed for 30mins and filtered through Whiteman GF/A paper by gravity. The beaker was rinsed with distilled water. The residue was washed on the paper with distilled water until the filtrate was neutral. The residue was transferred from the paper back to the beaker containing 200ml of hot 1.25% NaOH. Steps 4 and 5 were repeated. The paper with residue was transferred into a crucible, dried at 100°C overnight, cooled in a dessicator and reweighed (weight A). The samples were put in furnace at 600°C for 6h, cooled in a dessicator and reweighed (weight B). The loss in weight during incineration represents the weight of crude fibre.

$$\% \text{ crude fibre} = \frac{(\text{weight A}) - (\text{weight B})}{\text{Sample weight}} \times 100$$

2.4.4 Determination of fat (AOAC,) method [13,14]

Soxhlet system HT2 method was adapted. The sample was ground and dried and 2g were loaded in thimble and plugged with cotton wool. The thimble was dried and inserted into the Soxhlet HT. Extraction cups were dried and weighed (with boiling chips) and 25ml of the solvent was added into each cup. The cup was inserted into the soxhlet HT. The extraction cups were dried and weighed (with boiling chips). 25ml of the solvent was added to the solvent in each cup. The cup was inserted into the Soxhlet HT and extracted for 15mins in boiling position and for 30mins in "rinsing position". The solvent was evaporated; the cups were released and dried at 100°C for 30mins. The cups were cooled in a dessicator and reweighed.

$$\% \text{ fat/oil} = \frac{W_3 - W_2}{W_1} \times \frac{100}{1}$$

Where W_3 =weight of the cup with the extraction oil; W_2 =weight of the empty cup; 6.25=protein conversion factor; W_1 =Weight of the sample.

2.5 Determination of Protein

2.5.1 Digestion

Protein content was determined using micro-Kjeldahl method of AOAC [13,14]. About 1g of each sample was weighed into a 100ml micro-Kjeldahl digestion flask. About 1g of copper sulphate and 10g sodium sulphate were added to the flask and thoroughly shaken and placed on the digestion rack in an inclined position. The sample in the flask was digested by heating in a flame chamber until frothing ceased. The temperature was increased, allowed to boil for about one hour until the colour changed to bluish green. The clear digested sample was allowed to cool.

2.5.2 Distillation

Some distilled water was added to the digested sample with a wash bottle to 100ml in a 100ml volume metric flask. A-10ml of the digest was pipette and transferred into a micro-Kjeldahl distillation flask followed by the addition of 60ml of 60% sodium hydroxide (NaOH) solution. The flask was immediately fixed to the splash head of the distillation apparatus. A-4% boric acid was added into a 100ml receiving conical flask, 2 drops of methyl red indicator was added, in such a way that the outlet of the adapter of the delivery tube was extended under the surface of the boric acid solution. The mixture was heated to liberate ammonia into the receiving conical flask containing 100ml boric acid and the indicator until yellowish green colour distillate was obtained.

2.5.3 Titration

The distillate was titrated with 0.1N hydrochloric acid (HCl) until the end point of pink colouration was obtained. The percentage (%) protein was calculated thus:

$$\text{Protein (\%)} = \frac{T \times 0.0014 \times 6.25 \times 100}{\text{Wt of the sample}} \times 1$$

Where T = titre value of the sample
0.0014 = correction factor of the acid

2.6 Carbohydrate Determination

This was determined by difference method. The summation of all the proximate values was subtracted from 100%. Thus:

$$\% \text{ carbohydrate} = 100\% - (\% \text{ crude protein} + \% \text{ fat} + \% \text{ ash} + \% \text{ Crude fibre} + \% \text{ moistur})$$

2.7 Energy Determination

Energy was determined by the "Atwater factor". The energy value of the samples were calculated by multiplying the values for fat, CHO and protein with 17:37:17 the "Atwater factors" respectively.

$$\begin{aligned} \text{Where protein} &= 17\text{KJ/g} \\ \text{Fat} &= 37\text{KJ/g} \\ \text{CHO} &= 17\text{KJ/g} \end{aligned}$$

2.8 Vitamin Analysis

Pro-vitamin A (beta carotene) was determined using AACC method [15]. A 10g of each sample was weighed into a 250ml conical flask and 50ml of 50:50 acetones was added. This was allowed to stand for two hours (2h) with occasional shaking and filtering. The filtrate was measured and equal volume of saturated NaCl (50%) was added to wash the filtrate (i.e. carotene extract). The mixture was shaken, transferred to a separating funnel, and the layer of the extracted carotene was removed. The supernatant (upper layer) was washed again with equal volume of 100% potassium trioxocarbonate (IV) (K_2CO_3), separated and finally washed with about 10-20ml of distilled water. Water carotene was separated and the extracted carotenoid (either beta carotene or lycopene) was collected. The absorbance was read in a spectrophotometer at 326nm wavelength using 50:50 acetone low boiling petroleum ether solution as blank. The pro-vitamin A content was calculated as follows:

$$\text{Potency (units/g)} = 1900 \times E_{1cm}^{1\%} \text{ at } 328\text{nm}$$

The following correction was applied if the maximum lies in the same range, but the relative extensions are not within 0.02.

$$E_{328} \text{ (Corrected)} = 3.52 (2 E_{328} - E_{316} - E_{340})$$

2.9 Mineral Analysis

The method described by Ranjiham and Gapal [16] was used to determine calcium, iron and zinc. About 1g of each sample was weighed into 100ml round bottom flask. Five millilitres (5ml) of perchloric acid was added and heated over electric heater in a fume chamber until the solution became colourless. Each of the solution was made up to 10ml mark with distilled water and the diluted sample was set aside for further studies. The iron, zinc and calcium were analyzed using absorption spectrophotometric method.

3. RESULTS

Table 1 presents the proximate and energy composition of food materials (Maize, soybean and *Moringa oleifera* leaves) used. Moisture levels were 8.84% in *Moringa oleifera* leaves, 10.26% in maize and 6.66% in soybean. The crude protein level was 36.46% in soybean, 27.45% and 10.22% in *Moringa oleifera* leaves and maize respectively. Fat (ether extract) was 21.07% in Soybean, 3.47% in maize and 2.27% in *Moringa oleifera* leaves. Ash values ranged from 7.85% in *Moringa oleifera* leaves and 4.22% in soybean while maize had 1.80%. Crude fibre was 19.24% in *Moringa oleifera* leaves, 5.06% in soybean and 1.98% in maize. Carbohydrate was 72.28% in maize, 34.34% and 26.44% in *Moringa oleifera* leaves and soybean, respectively. The energy level was 1,849KJ in soybean, 1,531KJ in maize and 1,134KJ in *Moringa oleifera* leaves.

Table 2 shows the minerals and β -carotene contents of maize, soybean and *Moringa oleifera* leaves (%). Calcium was 1,335mg in *Moringa oleifera* leaves, 14.49mg in soybean and 11.67mg in maize. *Moringa oleifera* leaves had 26.44mg iron, soybean had 9.65mg and maize had 5.95mg. Zinc level was 7.49mg in *Moringa oleifera* leaves, 3.46mg in soybean and 1.87mg in maize. β -carotene was 3,846.15RE in *Moringa oleifera* leaves, 538.46RE in soybean and 102.56RE in maize.

Table 1. Proximate and energy composition of maize, soybean and *Moringa oleifera* leaves (%)

Nutrients	Maize	Soybean	<i>Moringa oleifera</i> leaves
Moisture	10.26±0.01	6.66±0.02	8.84±0.01
Crude protein	10.22±0.03	36.46±0.01	27.45±0.03
Fat	3.47±0.01	21.07±0.02	2.27±0.01
Ash	1.80±0.02	4.32±0.03	7.85±0.01
Crude fibre	1.98±0.01	5.06±0.01	19.24±0.02
CHO	72.28±0.02	26.44±0.02	34.34±0.01
Energy (KJ)	1,531±0.5	1,849±1.28	1,134±0.50

Mean±SD of three determinations

Table 2. Minerals and β -carotene contents of maize, soybean and *Moringa oleifera* leaves (mg/100g)

Nutrient	Maize	Soybean	<i>Moringa oleifera</i> leaves
Calcium	11.67±0.01	14.49±0.04	1,335±0.10
Iron	5.95±0.02	9.65±0.01	26.44±0.04
Zinc	1.87±0.01	3.46±0.01	7.49±0.02
β -carotene (RE)	102.56±0.00	538.46±0.00	3,846.15±0.00

Mean±SD of three determinations

4. DISCUSSION

4.1 Proximate and Energy Composition of Maize, Soybean and *Moringa oleifera* Leaf

The protein level of maize (10.22%) was higher than reported by Ihekoronye and Ngodi [17]. This could be as a result of maize variety and fermentation of the sample. During fermentation, there is microflora hydrolysis of protein into free amino acids (AAs) and subsequent synthesis of free AAs to new protein [18]. The protein content of soybean (36.46%) was similar to that recorded by George (36.50%) [19]. It is equally similar to that reported by Osho (40.00%) [20,21] and Ene-Obong and Carnovale (43.00%) on dry matter basis (39.06%) [22]. This is high because soybean is one of the richest natural food sources of protein. The protein content of *Moringa oleifera* leaves (27.45%) was similar to that recorded by Fuglie 27.1% [23]. However, it was higher than that observed by Okoyeh and Obizoba (16.75%) [24]. The high protein level could be due to method of processing.

The 21.07% ether extract of soybean confirms that it is an oil seed. Food and Agricultural Organization documented that soybean seed was the largest single producer of edible oil and account for roughly 50% of the total oilseed production of the world [25]. Maize has 3.47% fat which is close to 4.6% reported by Ihekoronye and Ngoddy [20,21]. *Moringa oleifera* leaves with 2.27% fat content were normal because leafy vegetables are known to be poor sources of fat [26].

The high fibre (19.24%) of *Moringa oleifera* leaves is of interest because fibre is important for effective digestion. Research by different groups recorded similar high fibre levels for *Moringa oleifera* leaves [23,24,27]. The fibre level of maize (1.98%) was in line with that reports [17,28] This low level of crude fibre of maize could be as a result of fermentation which decreases the crude fibre value of plant food products [29]. This is due to increased

hydrolytic activities of enzymes inherent in the grains which hydrolyze complex carbohydrate to increase the level of true sugars [30]. Soybean had 5.06% fibre which is lower than that reported by George (9.3%) [19]. The low fibre content of processed soybean is an advantage for the formulation of low fibre infant complementary food. This is because bulk would be reduced and nutrient intake increased. This is particularly important because of the small stomach capacity of the infants.

The high (7.85%) ash content of *Moringa oleifera* leaves was suggestive of high mineral content. This is in line with Fuglie who noted that Moringa family is rich in a number of minerals [23]. Soybean also had high (4.32%) ash level, which is indicative of high mineral content. The result supports George who documented that the soybean is possibly the richest natural food that exists in protein, minerals and vitamins [19]. The ash content of maize (1.80%) is similar to that reported by Onuoha and Okeke (1.24%) and Ihekoronye and Ngoddy (3%) [17,28].

The high carbohydrate content of maize (72.28%) was not a surprise. Maize is a staple (cereal) high in carbohydrate. The carbohydrate level of soybean is low (26.44%) because it is an oil seed with high protein level. *Moringa oleifera* leaves had moderate carbohydrate level as a result of drying which concentrated nutrients. This led to high energy level (1134KJ). The high energy level (1849KJ) of soybean in this study was probably due to high fat content of the seed. This is of advantage to underweight children and even wasted adults. Soybean could also be used to boost the energy intake of infants and individuals who are critically ill.

4.2 Mineral and B-carotene of Maize, Soybean and *Moringa oleifera* Leaves

Soybean and maize had low calcium (14.49 & 11.67mg), moderate iron (9.65 & 5.95mg) and zinc (3.46 and 1.87mg) and appreciable quantity of β -carotene (538.46 & 102.56%). The calcium level of *Moringa oleifera* leaves was as high as 1335mg/100g. It was close to 2,003mg/100g observed by Fuglie [23]. The tree for life organization noted that "Ounce for ounce" (gramme for gramme); Moringa leaves contain more vitamin A than carrots, more calcium than milk, more iron than spinach, more vitamin C than oranges, and more potassium than banana", and that the protein quality of Moringa leaves rivals that of milk and egg [31]. The high calcium content of Moringa leaves makes it suitable for infant complementary food since infants need calcium for bone and teeth formation. The iron was high (26.44%), zinc significant (7.49%) and β -carotene very high (3846.15RE). Fahey noted that the *Moringa oleifera* tree is extraordinary in that all parts of the tree are edible and has exceptionally high nutrient content [32].

5. CONCLUSION

Incorporation of pulverized *Moringa oleifera* leaves in infants' food could diversity food intake, ensure food security and reduce some micronutrient deficiency diseases. *Moringa oleifera* leaves processing and utilization through nutrition education should be encouraged amongst mothers and caregivers. *Moringa oleifera* tree cultivation around homes would ensure availability all year round to reduce micronutrient disease during fruits and vegetables off season.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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