

Effect of *Moringa oleifera* Leaf Powder Supplementation on the Micronutrient and Toxicant Contents of Maize – Soybean – Peanut Complementary Food Formulations

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Abstract: The effect of *Moringa oleifera* leaf powder supplementation on the micronutrient and toxicant composition of maize – soybean – peanut food formulations was determined. Maize, soybeans and peanut (MSP) flours were blended in a ratio of 60:30:10 (through material balancing to give 16g protein/100g) food as recommended by the protein advisory group (PAG) for infant diets. While one part was used unfortified as control food sample (MSPA), the remaining three parts were fortified with 5% (125g), 10% (250g) and 15% (375g) *Moringa oleifera* powder, giving samples MSPB, MSPC and MSPD respectively. Standard methods of analysis were then used to determine the content of some representative vitamins, amino acids, minerals and antinutritional factors in the food formulations. There was significant increase ($p < 0.05$) in Vitamins A (β -Carotene) and C as well as amino acids lysine and tryptophan with increase in *Moringa oleifera* powder, with values ranging from 2.40 to 5.43mg/100g, 2.00 to 3.80mg/100g, 34 to 145mg/100g and 13 to 45mg/100g for β -Carotene, Vitamin C, Lysine and Tryptophan respectively. Supplementation also significantly ($p < 0.05$) increased the contents of all the mineral elements with values ranging from 498.90 to 631.72mg/100g, 81.10 to 110.94mg/100g, 1.40 to 5.48mg/100g, 39.20 to 356.67mg/100g and 38.70 to 77.60mg/100g and 1.59 to 2.38mg/100g for potassium, magnesium, iron, calcium, phosphorus and zinc respectively; while oxalates, phytates and phenols contents increased significantly ($p < 0.05$) with increase in *Moringa oleifera* leaf powder addition, with values ranging from 70.42 to 84.80mg/100g, 68.00 to 90.04mg/100g and 80.00 to 83.40mg/100g respectively.

Keywords: *Moringa*, Leaf powder, Supplementation, Micronutrients, Toxicants, Food formulations.

INTRODUCTION

Micronutrient deficiencies/ malnutrition *i.e.* deficiencies of vitamins and minerals constitute the most wide spread form of malnutrition, with women and children being particularly vulnerable. It is a major impediment to socioeconomic development and contributes to a vicious cycle of underdevelopment, to the detriment of the already underprivileged groups. It has long – ranging effects on health, learning ability and productivity. It leads to high social and public costs, reduced work capacity in populations due to high rates of illness and disability, as well as tragic loss of human potential [1]. Poverty, lack of access to a variety of foods, lack of knowledge of optimal dietary practices and high incidence of infectious diseases are some of the factors which lead to micronutrient malnutrition. In growing children, the adverse effects of micronutrient deficiencies include: poor growth and development, mental and neuromotor performance, immunocompetence, physical working capacity, overall reproductive performance as well as increased morbidity, mortality and risk of maternal death [2].

More than two billion people in the world suffer from various micronutrient deficiencies. In Nigeria, the proportion of people suffering from micronutrient deficiencies and resultant diseases is at a critical level. For example, nearly 20 million Nigerians are estimated to suffer from iodine deficiency disorder (IDD), with the prevalence of goiter put at 20%; while the prevalence of nutritional anaemia, as a result of iron deficiency, is put at 25% among mothers and children and accounts for the very high maternal mortality in the country [3]. Overcoming micronutrient malnutrition is therefore, a precondition for ensuring rapid and appropriate national development.

A good number of global goals have been established and considerable investments have been made by governments and aid agencies in programmes designed to prevent micronutrient malnutrition in Nigeria and other developing countries. Different strategies have been employed, such as school lunch programmes, nutrition education, introducing exotic vegetables and even campaigns to give children massive doses of vitamin A. One of the major problems with many of these approaches is the dependence on imported solutions and outside personnel. Progress quickly stops, once the programme funding dries up [4]. The need to address this problem from a more holistic multidisciplinary

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approach through careful selection of locally available, inexpensive raw food materials is therefore, imperative.

The easiest and cheapest way of overcoming micronutrient malnutrition for the vast majority of the vulnerable groups of the population is the blending of our locally available food crops for the production of high nutrient, inexpensive and popular food products with high quantity/quality of micronutrients, to provide alternatives to the more expensive imported foods. The leaves, seeds and flowers of *Moringa oleifera* all have great nutritional and therapeutic value. The seeds are eaten like peas or roasted like nuts; while the flowers are eaten when cooked and taste like mushrooms. The leaves are outstanding as a source of vitamins A, B group and (C when raw) and are among the best sources of minerals. They are also excellent sources of protein, but poor sources of carbohydrates and fats. Thus, *Moringa* leaves are one of the best plant foods available in nature [5]. A maize – soybean – peanut food formulation is a high nutrient food rich in carbohydrates, fats and proteins, which can be used as a complementary food. Supplementation with *Moringa oleifera* flour, which is reputed to be very rich in micronutrients [4-8] is expected to further improve the quality of the food product.

Though these crops have various levels of anti – nutritional factors (especially the very high levels of oxalates reported for *Moringa oleifera*), it is expected that processing will reduce them to acceptable levels. Moreover, the low percentage of supplementation to be used (highest 15%) is not expected to increase the level of toxicants beyond safe levels.

The aim of this study was therefore, to investigate the effect of *Moringa oleifera* leaf powder supplementation on the micronutrient and toxicant composition of maize – soybean – peanut food formulations for possible use as complementary food.

MATERIALS AND METHODS

Materials Procurement

About 7.0kg of yellow maize (*Zea mays*, TZSR-Y); 4.0kg of soybeans (*Glycine max*, TGX 536-OZD) and 2.0kg peanuts (*Arachis hypogea*, QPG); 2009 harvest year seeds were purchased from Benue State Agricultural and Rural Development Authority (BNARDA), Makurdi; while 3.5kg of fresh *Moringa oleifera* leaves was obtained from a plant opposite Special Science Secondary School, University of Agriculture Road, Makurdi.

Material Preparation

Maize, Soybeans and Peanut flours were prepared using the method described by [9]. The clean yellow maize, soybeans and peanut were separately washed in clean tap water. The maize and peanut were air dried for 12 hours. The soybeans were soaked in tap water for 12hours and washed by rubbing between the palms to remove testa, then washed again several times with more water until most of the testa were washed out. It was then boiled for 15minutes in water, air dried for 48 hours and then dried in an oven at 70°C for 30minutes. (Soaking and roasting were intended to remove the beany flavour). The peanut was also roasted in an oven at 70°C for 30minutes and the seed coat removed to get clean partially roasted peanuts. The maize and the processed soybeans were separately milled in a disc attrition mill (ASIKO All, Addis, Nigeria) and sieved using a 500µm sieve. Peanut flour was obtained by pounding the roasted peanut in a wooden mortar for size reduction and milling in a 2L mistral grinder (model SAISHO, S – T4PN) into a smooth powder.

Moringa oleifera leaf powder was prepared using a modification of the method described by [8]. *Moringa* leaves were washed in clean tap water containing 5% Sodium chloride. They were then dried indoors at room temperature for four (4) days, grounded into powder and sieved with a fine sieve (500µm). The powder was then dried to constant weight in a hot air oven at 60°C. All flours were packaged in black polyethylene bags and stored in airtight plastic containers away from light.

Food Product Formulation/Supplementation

Maize, soybeans and peanut (MSP) flours were blended in a ratio of 60:30:10. This ratio was arrived at, based on their protein content through material balancing [10] to give 16g protein/100g food as recommended by the protein advisory group [11] for infant diets, as described by [8]. The blends were mixed thoroughly using a Kenwood mixer and then divided into four parts of 2.5kg each. While one part was used unfortified as control food sample (MSPA), the remaining three parts were fortified with 5% (125g), 10% (250g) and 15% (375g) *Moringa oleifera* powder, giving samples MSPB, MSPC and MSPD respectively. The fortified food formulations were thoroughly mixed separately using Kenwood blender and individually packaged in black cellophane bags and stored in air tight plastic containers in a cool dry place away from direct sun light, from where samples were taken for the different analyses.

ANALYSES

Micronutrients

While vitamin A was determined by the adaptation of the method described by [12] where the value of β – carotene obtained was divided by 6 to get a rough estimate of vitamin A); Vitamin C (Ascorbic acid) content was determined by the titrimetric method of [13]. Quantitative determination of some amino acids (lysine and tryptophan) was carried out using the method described by [14]. Mineral content in the food formulations was determined using Atomic Absorption Spectrophotometer (AAS model UNICAM 969 Solar) as described by [14].

Toxicants

The content of anti – nutritional factors (Phytates, Oxalates and Total phenols) were determined using the Isocratic High Performance Liquid Chromatography (HPLC) as described by [15].

Statistical Analysis

Data collected was subjected to analysis of variance (ANOVA) using a prepackaged computer software (MINITAB 15).

RESULTS

Vitamins and Amino Acids

Table 1 shows the effect of supplementation on the vitamin and amino acid content of the food formulations. There was a significantly increase ($p < 0.05$) in Vitamins A and C as well as amino acids lysine

and tryptophan with increase in *Moringa oleifera* powder, with values ranging from 2.40 to 5.43mg/100g, 2.00 to 3.80mg/100g, 34 to 145mg/100g and 13 to 45mg/100g for β -Carotene, Vitamin C, Lysine and Tryptophan respectively.

Minerals

The effect of supplementation on the mineral content of the food formulations is presented in Table 2. The mineral content increased significantly ($p < 0.05$) with increasing levels of supplementation, with values ranging from 498.90 to 631.72mg/100g, 81.10 to 110.94mg/100g 1.40 to 5.48mg/100g, 39.20 to 356.67mg/100g and 38.70 to 77.60mg/100g and 1.59 to 2.38mg/100g for potassium, magnesium, iron, calcium, phosphorus and zinc respectively.

Toxicants

The effect of supplementation on pH/anti-nutritional factors is presented in Table 3. Oxalates, phytates and phenols contents increased significantly ($p < 0.05$) with increase in *Moringa oleifera* leaf powder addition, with values ranging from 70.42 to 84.80mg/100g, 68.00 to 90.04mg/100g and 80.00 to 83.40mg/100g respectively.

DISCUSSION

Vitamins and Amino Acids

The increase in vitamin content is in agreement with the work of [16] and could be as a result of substitution effect, since *Moringa* leaves are reported to have very high quantities of vitamins A, C and B group as well as a good balance of all the essential amino acids [6].

Table 1: Vitamin and Amino Acid Content of Maize-Soybean-Peanut Food Formulations Fortified with *Moringa oleifera* Leaf Powder

Parameters	MSPA	MSPB	MSPC	MSPD	LSD	RDA
VitaminA (mg/100g)	2.40 ^d ±0.01	3.61 ^c ±0.01	4.82 ^b ±0.01	5.43 ^a ±0.02	0.23	1.5
VitaminC (mg/100g)	2.00 ^d ±0.02	2.80 ^c ±0.01	3.50 ^b ±0.02	3.80 ^a ±0.02	0.03	20
Lysine (mg/100g)	34 ^d ±0.82	78 ^c ±1.02	105 ^b ±1.20	145 ^a ±0.95	1.04	832
Tryptophan (mg/100g)	13 ^d ±0.62	24 ^c ±0.51	32 ^b ±0.75	45 ^a ±0.81	0.83	169

Values are means \pm standard deviations of triplicate determinations
Means with the same superscript within the row are not significantly different ($p > 0.05$).

Key:

MSPA= Maize, Soybean, Peanut diet
MSPB = Maize, Soybean, Peanut +5% *Moringa* powder
MSPC = Maize, Soybean, Peanut +10% *Moringa* powder
MSPD = Maize, Soybean, Peanut +15% *Moringa* powder
LSD = Least Significant Difference
RDA = Recommended Dietary Allowance for 1 – 3 year olds [16].

Table 2: Mineral Content of Maize-Soybean-Peanut Food Formulations Fortified with *Moringa oleifera* Leaf Powder

Parameters	MSPA	MSPB	MSPC	MSPD	LSD	RDA
Potassium (mg/100g)	498.90 ^d ±0.02	543.17 ^c ±0.03	587.45 ^b ±0.02	631.72 ^a ±0.02	0.04	800
Iron (mg/100g)	1.40 ^d ±0.01	2.76 ^c ±0.01	4.12 ^b ±0.01	5.48 ^a ±0.03	0.03	10
Magnesium (mg/100g)	81.10 ^d ±0.01	91.05 ^c ±0.03	100.99 ^b ±0.02	110.94 ^a ±0.02	0.03	150
Calcium (mg/100g)	39.20 ^d ±0.01	111.69 ^c ±0.03	224.18 ^b ±0.01	356.67 ^a ±0.03	0.04	400
Phosphorus (mg/100g)	38.70 ^d ±0.02	51.69 ^c ±0.01	64.63 ^b ±0.02	77.60 ^a ±0.02	0.03	800
Zinc (mg/100g)	1.59 ^d ±0.02	1.92 ^c ±0.02	2.09 ^b ±0.01	2.38 ^a ±0.01	0.03	3.9

Values are means ± standard deviations of triplicate determinations

Means with the same superscript within the row are not significantly different ($p > 0.05$)

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LSD = Least Significant Difference

RDA = Recommended Dietary Allowance for 1 – 3 year olds [16]

Table 3: pH/Anti-Nutrients of the Maize-Soybean-Peanut Food Formulations Fortified with *Moringa oleifera* Leaf Powder

Parameters	MSPA	MSPB	MSPC	MSPD	LSD
Oxalates (mg/100g)	70.42 ^d ±0.81	82.50 ^c ±0.71	83.63 ^b ±1.02	84.80 ^a ±0.92	0.60
Phytates (mg/100g)	68.00 ^d ±1.00	78.00 ^c ±0.91	86.00 ^b ±0.82	90.04 ^a ±1.02	1.00
Phenols (mg/100g)	80.00 ^d ±0.72	81.00 ^c ±1.01	82.00 ^b ±0.78	83.00 ^a ±0.83	0.74

Values are means ± standard deviations of triplicate determinations

Means with the same superscript within the row are not significantly different ($p > 0.05$).

Key:

MSPA: Maize, Soybean, Peanut diet

MSPB: Maize, Soybean, Peanut +5% *Moringa* diet

MSPC: Maize, Soybean, Peanut +10% *Moringa* diet

MSPD: Maize, Soybean, Peanut +15% *Moringa* diet

LSD: Least Significant Difference

Olushola [7] reported that fresh *Moringa oleifera* leaves contains four times the vitamin A (β - carotene) in carrots (6,780mg as against 1,890mg/100g) and seven times the vitamin C in oranges (220mg as against 20mg/100g); while Fulgie [6] reported Vitamin A (β - carotene) content of 18.90mg/100g and Vitamin C content of 17.30mg/100g for *Moringa oleifera* leaf powder.

Vitamin A is essential for growth and metabolism of all body cells. It is required for formation of rhodopsin (visual purple), a complex substance formed from retinol and protein. Rhodopsin is a pigment found in the retina, a membrane at the back of the eye, and is necessary for vision in reduced light. Vitamin A is also essential for the maintenance of a healthy skin and surface tissues, particularly the moist mucous membranes, such as the cornea at the front of the eye and the lining of the respiratory tract. Deficiency leads

to stunted growth, night blindness and reduced resistance to infection due to poor condition of the mucous lining of respiratory tract. In extreme cases, the tear glands become blocked and the membranes at the front of the eyes become dry and inflamed – 'Xerophthalmia'. Severe and prolonged deficiency can lead to ulceration of the cornea, causing blindness. Vitamin A content for all the food formulations in this study met the Recommended Daily Allowances (RDAs) for 1 – 3 year olds as given by [17].

Vitamin C is necessary for formation of collagen, the main protein of the connective tissue; which is the packaging material that separates, protect and support various organs. It also aids in the absorption of iron from the intestine. While most other animals synthesize their ascorbic acid from glucose in their body cells, only man, monkeys and guinea pigs require a dietary source of vitamin C. Deficiency of vitamin C leads to a

condition known as 'Scurvy' whose main symptoms are bruising and spontaneous haemorrhaging under the skin. The gums become black and spongy; wounds and fractures fail to heal on time. All these symptoms are caused by the failure to form connective tissue. Anaemia also occurs as a result of failure to absorb iron and inability to form red blood cells. Though there was significant increase ($p < 0.05$) in vitamin C, the food formulations did not meet the RDAs for 1 – 3 year olds as given by [17].

The significant increase ($p < 0.05$) in the lysine and tryptophan contents of the food formulations with increase in *Moringa oleifera* leaf powder supplementation could be due to the high levels of these amino acids in the powder. This is in agreement with [6] who reported values of 1,325mg/100g and 425mg/100g for lysine and tryptophan respectively for the leaf powder. Amino acids make the specific proteins required by the body's specialized tissues. Although the body is able to produce most of the amino acids it needs, several are not produced in sufficient quantities and must be obtained from the diet. These are called essential amino acids, and are all present in *Moringa oleifera* leaves, thus giving very high quality protein. This makes *Moringa oleifera* leaf powder a very good supplement for cereal flours which are poor in lysine and tryptophan, as well as for legume flours which are poor in sulphur containing amino acids.

Results in this work, however, show that the RDAs [17] for these amino acids for 1 – 3 year olds were not achieved at this level of supplementation.

Minerals

The increase in mineral content could be due to substitution effect, owing to the high mineral content of *Moringa* leaf powder as reported by many researchers [7, 8]. Fuglie [18] reported a calcium content of about 2003.00 mg/100g, potassium of 1,324.00 mg/100g, iron of 28.20mg/100g, magnesium of 368.00 mg/100g, phosphorus of 204.00 mg/100g and zinc content of 6.00mg/100g for *Moringa oleifera* leaf powder

Though supplementation significantly ($p < 0.05$) increased the contents of all the mineral elements, they did not attain their RDAs as given by [17]. Minerals are vital to the functioning of many body processes. They are critical players in nervous system functioning, other cellular processes, water balance, and structural (e.g. skeletal) systems. Calcium builds healthy/strong bones and teeth and also assists in blood clotting [19]. Calcium intake is very essential during childhood growing years. Deficiency can cause rickets, bone pain

and muscle weakness. Potassium helps the body to maintain normal water balance in cells, transmit nerve impulses, keep acids and alkalis in balance and stimulate normal movement of the intestinal tract [19]. Potassium deficiency can cause vomiting, acute muscle weakness and loss of appetite. Magnesium helps the body to maintain and repair cells and also provides energy. Deficiency can result in weakness, tiredness, vertigo, convulsions, nervousness, cramps and heart palpitations. Iron is a vital component of red blood cells, which carry oxygen. It assists the muscles to keep reservoirs of oxygen and makes the body more resistant to infections. Iron deficiency can cause anemia, tiredness, headache, insomnia and heart palpitations; while deficiency in children can cause stunted growth and impaired mental capability [20]. Phosphorus provides energy and helps build the structure of bones and teeth. Deficiency can lead to loss of appetite, weakness, bone pain and mental confusion. However, phosphorus deficiency is rare because it is present in many foods [7].

Toxicants

The increase in oxalates could be due to the high oxalate content of *Moringa oleifera* leaf powder and is in conformity with the findings of other researchers. Fuglie [18] reported oxalate content of about 1,600.00mg/100g. Apart from causing irritation oxalate forms insoluble complexes with some metals, especially calcium and iron, thus leading to reduced calcium and iron availability/ metabolism [21]. Bender [22] however, reported that the risk of calcium deficiency diseases due to consumption of oxalate-rich foods is very rare. This is because humans are able to efficiently utilize very low amounts of calcium in their food [23]. Therefore, only consumption of very large amounts of oxalic acid is liable to cause calcium deficiency [18].

Phytic acid is the primary phosphate reserve in most seeds accounting for about 60 to 90% of total phosphorus. Phytate forms insoluble salts with metals such as calcium, iron, zinc and magnesium, thus rendering them unavailable for absorption in the body. It also adversely affects protein and starch digestibility [24]. Though the smallest 'toxic' dose of phytate in humans is not known, it appears that high doses are required for any appreciable effect [25].

Total phenols inhibit the activity of digestive and hydrolytic enzymes, such as beta-amylase, trypsin and lipases; thus decreasing the availability of reducing sugars, vitamins and minerals [14]. Information on dietary intake of polyphenols is rare; only a few

estimates are available: 1.0g/day (USA), 23mg/day (Dutch) and 28mg/day (Denmark) [26]. Considering that some cereals and vegetables, which are regularly consumed without any visible adverse effects, contain as much as 400mg/100g and 2000mg/100g phytate respectively [26], the levels in these food formulations (68.00 – 90.04mg/100g) are regarded as safe.

CONCLUSION

Supplementation with up to 15% *Moringa oleifera* leaf powder significantly improved the micronutrient content of Maize – Soybeans – Peanut food formulations, in some cases even meeting the Recommended Daily Allowances (RDAs) for 1 -3 year old children. Though the content of natural toxicants also significantly increased, the levels were considered safe, going by the levels reported for other staple foods. The food formulations could therefore be used as complementary foods to help alleviate malnutrition, especially in the poor developing countries.

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