Micronutrient and Antinutritional Content of Weaning Food Produced from Blends of Millet, Soya Beans and Moringa Oleifera Leaf Flour

Jane Hembadoon Gwer, Bibiana Dooshima Igbabul and Simon Terver Ubwa

Abstract — Any Weaning food offered to infants should be rich in micronutrients and high in protein quality. The study assessed the Mineral, Vitamin A and C, Amino acids and Antinutritional profile of weaning food blends from millet flour (MLF), soya beans flour (SBF) and moringa oleifera leaf flour (MLF) in the ratio FMF:SBF:MLF- sample C (60:35:5), sample D (60:30:10), sample E (60:25:5), sample F (60:20:20) sample A (100 % FMF) was used as control, sample B (FMF(60):SBF(40)). All analyses were done using standard methods. The Mineral, Vitamin A, C and Amino acid Tryptophan contents of the weaning food formulations significantly ($p<0.05$) increased with increased MLF Percentage. The Mineral values ranged from 57.37 to 466.87 mg/100g, 11.33 to 107.30 mg/100g, 36.27 to 62.38 mg/100g, 2.45 to 4.77 mg/100g and 76.64 to 178.09 mg/100g for calcium, iron, potassium, zinc and sodium respectively. Vitamins A (β -Carotene) and C ranged from 0-5.25 and 0.58-3.89 mg/100g respectively. Tryptophan ranged from 1.16-2.40 g/100g protein. Manganese, Lysine, methionine was significantly ($p<0.05$) higher in MLF unsubstituted sample B. The anti-nutrients analyzed were significantly ($p<0.05$) low with values ranging from (0.09 to 0.43) mg/100 g, (0.13 to 1.49) mg/100 g, (0.33 to 0.95) mg/100 g and (0.03 to 0.07) mg/100 g for phytates, tannins, oxalates and trypsin inhibitor respectively.

Index Terms — Amino acids, Antinutrients, Micronutrients, Weaning Food.

I. INTRODUCTION

In Africa, children are usually weaned with porridges made from either of the cereals such as maize, millet, sorghum or wheat. Cereals are the major dietary energy suppliers and provide significant amount of fat, protein, minerals (potassium and calcium) and B vitamins [1]. The cereal grains such as millet, provide more than 70 % of calories for the majority of poor people in the developing world [2]. Cereal products are limiting in some essential amino acids which make them to have poor nutritional value [3]. Considering that porridges made from cereals are the primary weaning foods in West Africa, efforts has been made by several researchers in enriching the porridges with legumes which supply the protein lacking in cereals [4]. Millet protein is deficient in lysine and tryptophan but has fair amounts of sulphur-containing amino acids (methionine and cystine) [5]. Soya bean is an excellent raw material to improve the nutritional quality of millet-based products especially protein quality, because of its complete amino acids profile [6]. It is

however evident that cereals-legumes blend is low in micronutrients. Various sources of micronutrients have been proposed and utilized with the aim of fortifying weaning foods. However, a continuous search for a relatively cost effective and readily available plant has led to the discovery of Moringa plant. Moringa (*Moringa oleifera*) often referred to as miracle tree could be a solution to the making of a complete weaning food. This is because every part of it can be used for food, medication and industrial purposes [7]. Especially, the leaves of the tree is a potential source to improve micronutrients, boost food security and foster rural development. The tree could be a solution to the problem of weaning food in developing countries. Studies have shown that the leaves have immense nutritional values such as minerals, vitamins and amino acids and as a result, the leaves have been used to combat malnutrition especially among infants and nursing mothers [8], [9]. The substitution of millet and soya bean weaning food formulations with *Moringa oleifera* leaf flour can dramatically improve their protein and micronutrient quality [10]

II. MATERIALS AND METHODS

A. Study Area

The study area was Makurdi Metropolis of Benue State located within Latitude 7.7322° N and Longitude 8.5391° E with an estimated area of 41,035Km² and has a population put at 342 500 [11].

B. Sample Collection

Millet and Soya beans were purchased from Wurukum market, Makurdi in Benue state, while fresh *Moringa oleifera* leaves were obtained from *moringa oleifera* plants in the College of Food Technology, University of Agriculture, Makurdi.

III. SAMPLE PREPARATION

A. Preparation of fermented millet flour

Fermented millet flour was prepared using a modified method of Enujiugha [12]. High quality millet was carefully selected and sorted, then washed to remove dust and stones. The grains were then soaked for 24 h at ambient temperature of 28±3 °C and Relative humidity of 65±3 %, rinsed to get rid

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Jane Hembadoon Gwer, Benue State University Makurdi, Nigeria.
(e-mail: janegwer86@gmail.com)
Bibiana Dooshima Igbabul, Federal University of Agriculture Makurdi, Nigeria.
(e-mail: bibibul46@gmail.com)

Simon Terver Ubwa, Benue State University Makurdi, Nigeria.
(e-mail: drsimonterverubwa@gmail.com)

of the soaked water, wet milled using an attrition mill (Senwei GX160) and sieved with muslin cloth. The resultant slurry was fermented for 24 h at 30 ± 1 °C after which it was dewatered, then tied in a sack cloth and suspended to drain most of the free water for 12 h and then dried for 5 h at 60 °C in an oven (Genlab oven model ov/100/F). The dried fermented flour lumps were broken down and milled into finer flour particles using a hammer mill and passed through 0.25 mm sieve to achieve fine fermented millet flour.

B. Preparation of soya beans flour

Soya beans flour was prepared using the method described by Solomon [13]. The Soya beans were washed and soaked in distilled water for 8 h, at ambient temperature of 28 ± 3 °C. The soaked soya beans were cooked in boiling water for 20 minutes, then dehulled by washing and rubbing between the palms to remove testa, then washed again several times with more distilled water to remove the hulls, then drained on a flat sieve for 1 h. The boiled and dehulled beans were dried for 5 h at 60 °C, then roasted at 200 °C for 30 minutes in the oven (Genlab oven model ov/100/F). The roasted beans were allowed to cool and milled into flour (using a hammer mill), the flour was then passed through 0.25 mm sieve to achieve fine soya beans flour.

C. Preparation of *Moringa oleifera* leaf flour

Moringa oleifera leaf flour was prepared using a modified method of Shiriki et al. [14]. *Moringa oleifera* leaves were washed in clean tap water containing 5 % Sodium chloride. They were then drained, spread thinly on trays and dried indoors at room temperature (28 ± 3 °C) for 24 hours, transferred to the oven (Genlab oven model ov/100/F) and dried at 40 °C for 1 h, then milled into flour using Kenwood electric blender BL 440 and sieved with a fine sieve (0.25 mm) to achieve *moringa oleifera* leaf flour.

IV. PRODUCT FORMULATION

The treated flour samples of millets, soya beans and *moringa oleifera* leaves were formulated into six different weaning food samples. Exactly 100 % fermented millet flour (sample A) was used as the control. Fermented millet flour and soya beans flour were blended in the ratio 60:40 (sample B). Fermented millet flour, soya beans flour and *moringa oleifera* leaf flour were blended in the ratio 60:35:5 (sample C), 60:30:10 (sample D), 60:25:15 (sample E) and 60:20:20 (sample F). The proportions at different levels were mixed in a Kenwood mixer to achieve a uniform blending to produce the weaning food samples. They were carefully packaged and sealed in polythene bags and kept tightly covered in glass jars for further analysis.

V. ANALYSIS

Mineral content of the food formulations was determined using Atomic Absorption Spectrophotometer (AAS model UNICAM 969 Solar) as described by AOAC [15]. Vitamin A was determined by the adaptation of the method described by Alexander and Griffiths [16] 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

titrimetric method [17]. Quantitative determination of amino acids was carried out using the method described by Kaspar et al. [18] and Tryptophan was determined using the method described by Margit and Ibolay [19]. Anti – nutritional factors (Phytates, Oxalates and Tanins) were determined using the Isocratic High Performance Liquid Chromatography (HPLC) as described by Onwuka [20], and Trypsin inhibitors [21].

VI. STATISTICAL ANALYSIS

The data generated were statistically analyzed for means and standard deviation and Analysis of Variance (ANOVA) was used to test the level of significance. Duncan's New Multiple Range Test was used to compare and separate means. Significance was accepted at ($p < 0.05$). The charts were plotted using Microsoft Excel 2013 version.

VII. RESULTS

A. Mineral

The result of the mineral content of the weaning food samples is presented in the order A, B, C D, E and F. Calcium (Ca), Iron (Fe), Potassium (K), Zinc (Zn), Sodium (Na), Magnesium (Mg) and Maganese (Mn).

Fig. 1. Mineral composition of the formulated weaning food blends from millet soya beans and *moringa oleifera* leaf flour.

- A=100% Fermented millet flour (The Control);
- B=60% Fermented millet and 40% Soya beans flour;
- C=60% Fermented millet and 35% Soya beans and 5% *Moringa oleifera* leaf flour;
- D=60% Fermented millet and 30% Soya beans and 10% *Moringa oleifera* leaf flour;
- E=60% Fermented millet and 25% Soya beans and 15% *Moringa oleifera* leaf flour;
- F=60% Fermented millet and 20% Soya beans and 20% *Moringa oleifera* leaf flour.

Ca content of the MLF substituted samples increased significantly ($p < 0.05$) with increased MLF Percentage. Values obtained were (57.37 ± 2.40 , 271.70 ± 2.35 , 403.37 ± 3.01 , 425.27 ± 2.58 , 441.87 ± 1.74 and 466.87 ± 3.25) mg/100g. Iron (Fe) values obtained were (11.33 ± 0.20 , 60.83 ± 0.45 , 71.40 ± 1.08 , 84.60 ± 0.30 , 98.93 ± 0.35 and 107.30 ± 0.46) mg/100g. The potassium values obtained were (360.27 ± 0.04 , 600.63 ± 0.01 , 610.04 ± 0.01 , 617.56 ± 0.01 , 629.62 ± 0.02 and 641.38 ± 0.01) mg/100g. Zn values were (2.45 ± 0.01 , 4.18 ± 0.01 , 4.29 ± 0.01 , 4.54 ± 0.01 , 4.57 ± 0.01 to 4.77 ± 0.01) mg/100g. Sodium values were (76.64 ± 0.01 , 150.97 ± 0.01 , 161.08 ± 0.01 , 169.01 ± 0.01 , 172.79 ± 0.01 and 178.09 ± 0.00) mg/100g. Magnesium values

were (14.28±0.01, 16.76±0.01, 17.26±0.01, 17.52±0.01, 17.67±0.02 and 17.79±0.01) mg/100g with the control having the lowest and sample F with the highest.

The Manganese (Mn) values were (0.58±0.01, 2.53±0.01 2.16±0.01, 2.08 ±0.01, 1.95±0.01 and 1.83±0.01) mg/100g for samples A, B, C, D, E and F respectively. The Mn content was observed to be highest in sample B, lowest in sample A, and decreased significantly ($p<0.05$) in MLF substituted samples with increased MLF percentage.

B. Vitamin A and C

The result of Vitamin A (Beta Carotene) and Vitamin C content of the weaning food formulation from millet, soya beans and *moringa oleifera* flour is shown in Table I. *Moringa oleifera* flour substitution significantly ($p<0.05$) increased the Beta carotene and Vitamins C content of the formulated weaning food with increased MLF percentage, with values in the range 0.00 to 5.25 mg/100g and 0.58 to 3.89 mg/100g respectively.

TABLE I: BETA CAROTENE AND VITAMIN C CONTENT OF THE FORMULATED WEANING FOOD BLENDS

Sample	Beta carotene (mg/100g)	Vitamin C (mg/100g)
A	0.00 ^f ±0.00	0.58 ^f ±0.02
B	1.68 ^e ±0.06	0.77 ^e ±0.03
C	2.91 ^d ±0.04	2.10 ^d ±0.15
D	3.83 ^c ±0.03	2.84 ^c ±0.04
E	4.43 ^b ±0.03	3.31 ^b ±0.04
F	5.25 ^a ±0.02	3.89 ^a ±0.02

Values are means ± SD of triplicate determinations. Means within the same column bearing different superscript are significantly different ($p<0.05$).

C. Amino Acids

Figure 2 shows the result of essential amino acids profile of the formulated weaning food samples. The essential amino acid Tryptophan increased significantly ($p<0.05$) in MLF substituted samples with increased MLF percentage, with values ranging from 1.16 to 2.40 g/100g protein. Methionine, lysine threonine, isoleucine, leucine and histidine were observed to be lowest in the control, highest in sample B and decreased significantly ($p<0.05$) with increased MLF percentage, with values ranging from (2.01 to 3.06, 3.05 to 4.96, 2.88 to 3.94, 3.24 to 4.44 and 0.28 to 2.68) g/100g protein respectively. Phenylalanine and valine decreased significantly ($p<0.05$) with increased MLF percentage with values ranging from (2.08 to 3.51 and 3.15 to 4.85) g/100g protein respectively.

Fig. 2. Essential amino acids of the formulated weaning food from millet, soya beans and *moringa oleifera* leaf flour.

D. Anti-Nutrients

The anti-nutrients analyzed were significantly ($p<0.05$) low with values ranging from (0.09 to 0.43) mg/100g, (0.13 to 1.49) mg/100g, (0.33 to 0.95) mg/100g and (0.03 to 0.07) mg/100g for phytates, tannins, oxalates and trypsin inhibitor respectively. However, phytates was highest in sample A and decreased significantly ($p<0.05$) with increased MLF percentage. Tannin and Trypin inhibitor were highest in sample B and decreased significantly ($p<0.05$) with increased MLF percentage, while oxalate increased significantly ($p<0.05$) with increased MLF percentage.

TABLE II: ANTINUTRIENT PROPERTIES OF THE FORMULATED WEANING FOOD BLENDS

Samples	Phytates (mg/100g)	Tannins (mg/100g)	Oxalate (mg/100g)	Trypsin inhibitor (mg/100g)
A	0.21 ^{cd} ±0.02	0.13 ^f ±0.02	0.33 ^f ±0.03	0.03 ^e ±0.02
B	0.43 ^a ±0.02	1.49 ^a ±0.01	0.58 ^e ±0.04	0.07 ^a ±0.04
C	0.32 ^b ±0.02	1.12 ^b ±0.01	0.66 ^d ±0.03	0.07 ^a ±0.01
D	0.27 ^{bc} ±0.04	0.86 ^c ±0.01	0.76 ^c ±0.04	0.06 ^b ±0.02
E	0.17 ^d ±0.02	0.72 ^d ±0.02	0.85 ^b ±0.04	0.05 ^c ±0.03
F	0.09 ^e ±0.01	0.55 ^e ±0.01	0.95 ^a ±0.03	0.04 ^d ±0.01

Values are means ± SD of triplicate determinations. Means within the same column bearing different superscript are significantly different ($p<0.05$).

VIII. DISCUSSION

A. Mineral

The mineral content of the *Moringa oleifera* leaf (MLF) substituted samples contained substantial amounts of Ca, Fe, K, Zn, Mg and significantly ($p<0.05$) increased with increased MLF percentage. The observed increase in Ca, Fe, K, Zn, Mg contents of the weaning food formulations is in accordance with the work of Shiriki et al. [22]. Abioye and Aka [23] also reported an increased mineral content when Maize-ogi was supplemented with *moringa oleifera* leaf flour.

The Ca content in this study which was in the range 403.37 to 466.87 mg/100g compares favourably with the Recommended Dietary Allowance (RDA) of 400 mg for children between 0-3 years [24]. MLF at 5 % in the formulation is enough to meet up with the RDA [24]. Calcium is an essential micronutrient in infants and young children for building bones and teeth, functioning of muscles and nerves, blood clotting and for immune defense [25].

The Iron (Fe) content in the range 11.33 to 107.30 mg/100g significantly ($p<0.05$) increased with increased MLF percentage. The control sample compared favourably with the RDA, but MLF substituted samples were significantly ($p<0.05$) higher than the RDA for infants 7 to 12 months which is 11 mg [24]. It must be noted that iron provided by the formulated weaning food in this study is nonheme which is less bioavailable for human absorption unlike the iron from animal which is more bioavailable [26]. Iron in weaning foods is needed for the production of hemoglobin which carries oxygen in the blood [27].

Zinc (Zn) content was in the range 2.45 to 4.77 mg/100g. The Zn content of the MLF substituted samples were significantly ($p<0.05$) higher than the RDA for infants 0-1 year which is 3.9 mg/day [24]. The Zn content observed to

have increased upon substitution with MLF maybe as a result of high Zn content in *Moringa oleifera* leaves. Adding *moringa oleifera* leaf flour to cereal-legume blends has also been shown to improve the Zn and Fe content of weaning foods [28]. Zn is involved in cellular growth and differentiation. Zn has been shown to be an essential component or cofactor in the enzyme that converts provitamin A into retinol and its deficiency seems to interfere with Vitamin A metabolism [29].

Potassium (K) content of the formulated weaning food which was in the range 360.27 to 641.27 mg/100g is significantly ($p < 0.05$) lower than the RDA of 800 mg/day for children 1 to 3 years [24]. MLF at 20% in the formulations will provide up to 80% of the RDA. The significant increase of K in MLF substituted samples maybe due to the high content of K content in soya beans and *moringa oleifera* leaves [30]. A major function of potassium is helping 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 [31].

Magnesium (Mg) values obtained in this study in the range 14.28 to 17.67 mg/100g is significantly ($p < 0.05$) lower than the recommended daily intake (RDI) of 75 mg/day for infants 7–12 months [32]. Netshiheni [33] who worked on maize porridge fortified with *Moringa oleifera* leaves and termite reported values higher than the recommended daily intake. This disparity in the findings may be due to the fact that *Moringa oleifera* leaves have been reported to differ in nutrient composition at different locations [34].

The Sodium (Na) content in the range 76.64 to 178.09 mg/100g is significantly ($p < 0.05$) lower than the RDI of 200 mg/day for infants 7–12 months [35]. MLF at 20% substitution in the formulation will provide 89% of the RDI value.

B. Vitamin A and C

Vitamin A (Beta Carotene) was in the range 0.00 to 5.25 mg/100g. The absence of Beta carotene in sample A could be attributed to the processing methods applied during the production of fermented millet flour (Ogi) [36] which may have resulted to the nutrient being leached, as bulk of the water used during the processing was discarded. The significant increase ($p < 0.05$) in beta carotene content observed in MLF substituted samples with increased MLF is in agreement with the work of Shiriki et. al. [22] and Shar et. al. [37]. The increased beta carotene content may be as a result of substitution effect, since *moringa oleifera* leaves has been reported to have high quantities of beta carotene [38]. The values obtained in this study is significantly ($p < 0.05$) higher than the RDA of 1.5 mg/day [24], and significantly ($p < 0.05$) higher than 1.28 mg/100 g reported by Glover-Amengor et al. [39].

Vitamin C content was in the range 0.58 to 3.89 mg/100g. The Vitamin C content of sample A may be as a result of fermentation as it has been reported that fermentation increases the vitamin C content of foods [40]. Vitamin C content also significantly ($p < 0.05$) increased in MLF substituted samples with increased MLF leaf flour percentage. The higher vitamin C content of MLF substituted samples in this study with increased percentage might be attributed to their presence in the *Moringa oleifera* leaves

[33]. The values obtained in this study are significantly ($p < 0.05$) lower than the RDA of 20 mg/day, sample F will provide up to 19.45% of the RDA [24]. This implies that other sources of vitamin C will be needed by the infants weaned using these blends in other to boost the vitamin C intake. Only a varied diet guarantees the supply of micronutrients, enhances good eating habits, and prevents the development of anorexia caused by monotonous foods [41], [42].

C. Amino Acids

The essential amino acids of the formulated weaning food samples substituted with *moringa oleifera* leaf flour (MLF) were significantly ($p < 0.05$) higher than the control sample and compared favourably with the FAO/WHO reference values for weaning foods [43].

The essential amino acids methionine which is the limiting amino acids in legumes decreased significantly ($p < 0.05$) with increased MLF substitution. Methionine contents of Samples B and C can provide 87.43 and 86.29% respectively, the FAO/WHO reference of 3.5 [43]. The observed decrease in methionine contents of the food formulations with increased MLF percentage maybe as a result of lower levels of methionine in *Moringa oleifera* leaves. This agrees with the findings of Bundit and Toshiro [44] that replacing *moringa oleifera* leaf partially by protein replacement in soya bean meal of fancy carp at increasing percentage decreased the methionine contents in the supplemented diets [44].

Tryptophan values increased significantly ($p < 0.05$) in MLF substituted samples with increased MLF percentage. This may be attributed to the substitution effect of MLF. This is in agreement with the work of Shiriki et al. [22] who also reported an increased tryptophan content with increased MLF percentage.

Lysine contents were observed to decrease in MLF substituted samples with increased MLF substitution. Although, the formulated samples were still significantly ($p < 0.05$) higher than the control (sample A). The highest content of Lysine in sample B may be due to high percentage of soya beans in the formulation as soya beans is a high-protein legume and an excellent complement to lysine-limited cereal protein [45].

D. Anti-Nutrients

The phytate content in the range 0.19 to 0.21 mg/100g significantly ($p < 0.05$) decreased with increased MLF substitution. The observed decrease in phytate content with increased MLF percentage may be attributed to the low values of Phytate in MLF as reported by Kayi [38], and also may be attributed to the absence of seed coats as achieved by dehulling of the soya beans [46]. The phytate content observed to have decreased in this study is expected to enhance the bioavailability of proteins and dietary minerals such as iron, zinc, calcium and magnesium of the weaning food formulations [46]. Dietary phytate at low levels are said to have beneficial role as an antioxidant, anticarcinogens and are likely to play an important role in controlling hypercholesterolemia and atherosclerosis [47].

The oxalate content in the range 0.33 to 0.95 mg/100g significantly ($p < 0.05$) increased with increased MLF percentage. The concentration of oxalate found to be higher in MLF substituted samples may be as a result of large

amounts of oxalate found in the stems and leaves of *Moringa oleifera* leaves [48]. This is similar to the findings of Ewolu et al. [49] who also reported increased oxalate content with increased MLF percentage in the production of kokoro. The oxalate content in this study is considered to be safe as they are below the safe normal range of 4-9 mg/ 100 g for oxalates as reported by Siddhuraju and Becker [50].

The Tannin content in the range 0.13 to 0.55 mg/100 g significantly ($p < 0.05$) decreased with increased MLF substitution. The decreased tannin contents with increasing MLF percentage may be because tannin has been found negligible in all fractions of *moringa oleifera* plant [51]. This implies that Moringa leaves' tannins which is a protein-inhibitor are not significant and may not affect the protein content of the formulated weaning foods when consumed [46].

Trypsin inhibitor found to be in the range 0.03 to 0.04 mg/100 g. significantly ($p < 0.05$) decreased with increased MLF substitution. The highest content of trypsin inhibitor found in (sample B) may be as a result of high percentage of soya beans in the formulations, as high levels of trypsin inhibitor is found in raw soya beans [52] but the low levels found in this study may be as a result of the processing methods used such as soaking, dehulling and degradation caused by heat treatment such as boiling and roasting applied in the processing of the soya beans flour [46].

The anti-nutrient levels were generally low and thus may not pose an immediate effect on the health of consumers. However, their presence suggests that a steady consumption may lead to toxic levels [52].

IX. CONCLUSION

Processing technologies such as fermentation, dehulling, cooking, roasting and drying were applied to produce acceptable and improved nutrient quantity and quality micronutrient rich ready to use weaning food due to hydrolysis and degradation of complex food reserves to simpler absorbable molecules. The formulated weaning food particularly millet, soya bean and *moringa oleifera* leaf in the ratio 60:20:20 improved the micronutrient i. e. Minerals, Vitamins A and C and essential amino acids Tryptophan while sample B with millet and soya bean in the ratio 60:40 improved Lysine and Methionine. In some cases, even meeting the Recommended Daily Allowances (RDAs) for children 1 -3 year. The content of antinutrients also significantly ($p < 0.05$) decreased but oxalate increased, although the levels were considered low and safe. The food formulations could therefore be used as weaning foods to improve micronutrients and boost food security especially in rural areas.

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Jane Hembadoon Gwer was born in Gboko LGA of Benue state on the 30th of April, 1986. Obtained Bachelor of Science degree in food technology, from the University of Agriculture Makurdi, Benue State in 2011. A Master of Technology in food science and technology from the Federal University of Technology Akure, Ondo State in 2015. She is currently working as an ASSISTANT LECTURER in the department of Vocational and Technical Education at Benue State University Makurdi, Benue State. She has published articles such as “Nutritional and *In vitro* Glycemic Properties of Selected Indigenous Tubers”, *Asian Food Science Journal*, 2018” and Effect of substitution of soya beans and moringa oleifera leaf flour on the properties of a traditional weaning food”, *European Journal of Food Science and Technology*, 2020”. She does research in Food Nutrition and her current research is in Food processing and nutrition.

Prof. Mrs Bibiana Dooshima Igbabul was born in Vandeikya LGA of Benue state on the 15th of May, 1969. Obtained Bachelor of Science and Master of Science degree in food science and technology, at the University of Agriculture Makurdi, Benue State in 1994 and 2001 respectively. A Doctor of Philosophy in food processing technology from the University of Agriculture Makurdi, Benue State in 2007. She currently works as a professor in the Department of Food Science and Technology, University of Agriculture, Makurdi, Benue State. She has published articles such as “Proximate micronutrient composition, physical and sensory properties of cookies produced from wheat, sweet detar and moringa leaf flour blends. *Current Research in Nutrition and food Science Journal*, 2018. Quality Characteristics of Cookies Prepared from Wheat and Fermented Afzelia Africana. *American Journal of Food Science and Technology*, 2018. Physicochemical and Sensory Properties of Cookies Produced From Composite Flours of Wheat, Cocoyam and African Yam Beans, *Journal of Food Research*, 2015. She does research in food processing and her current research is in Food processing and Food nutrition.

Prof. Igbabul is a member of Nigerian Institute of Food Science and Technology (NIFST) and Institute of Food Technologists, USA.

Prof. Simon Terver Ubwa was born in Mkar, Gboko LGA of Benue state on the 14th September, 1966. Obtained Bachelor of Science chemistry, from the University of Calabar, Cross River State in 1991. Master of Science chemistry (organic chemistry) from the University of Ibadan in 1997. A Doctor of Philosophy in pure and industrial chemistry (petroleum chemistry) from the University of Nigeria, Nsukka, Enugu State in 2011.

He currently works as a professor in the Department of Chemistry, Benue State University, Makurdi. Simon does research in Food Chemistry, environmental organic chemistry and biofuels. His current project is 'nutrition'. He has published articles such as Quantitative estimation of hydrogen Cyanide in fresh and cooked tuber Parenchyma (Pulp) of three Cultivars of sweet Cassava Cultivars grown in some parts of Benue State, Nigeria. *Food and Nutrition Sciences*, 2015. Effects of Traditional Smoking Methods on the concentration of Polynuclear aromatic Hydrocarbons (PAHs) in some species of Smoked Fish Traded in Benue State. *Journal of Food Research*. 2015. Assessment of Heavy metals in blood and some selected entrails of cows, Goats and Pigs slaughtered at Wurukum Abbatoir, Makurdi-Nigeria. *Advanced Analytical Chemistry*, 2017.

He does research in Food Chemistry, Environmental Organic Chemistry and Biofuels. His current project is 'nutrition'.

Prof Ubwa is member chemical Society of Nigeria (MCSN), Institute of chartered Chemist of Nigeria (MICCON).