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Essential Mineral of the New Shoots of Palmyra Enriched with *Moringa oleifera* Leaves and *Vigna unguiculata* Bean Powders

Mahan Makado Romuald[1*], Deigna-Mockey Viviane[1], Coulibaly Adama[2], Sidibe Daouda[1] and Biego Godi Henri Marius[1,3]

[1]Laboratory of Biochemistry and Food Sciences, Training and Research Unit of Biosciences, Felix Houphouët-Boigny University, 22 BP 582 Abidjan 22, Côte d'Ivoire.
[2]Training and Research Unit of Biological Sciences, Peleforo Gon Coulibaly University, BP 1328 Korhogo, Côte d'Ivoire.
[3]Department of Public Health, Hydrology and Toxicology, Training and Research Unit of Pharmaceutical and Biological Sciences, Felix Houphouët-Boigny University, BP 34 Abidjan, Côte d'Ivoire.

Authors' contributions

This work was carried out in collaboration between all authors. Author BGHM supervised the whole investigation. Author MMR designed the study, performed the experiment and wrote the manuscript assisted with authors DMV and CA. Authors DMV and MMR performed the statistical analysis of the results and checked the revised manuscript. Authors CA and SD participated in interpretation of the results. All authors read and approved the final manuscript.

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ABSTRACT

The purpose of this study is to contribute to a better valorization of Palmyra (*Borassus aethiopum*) by the content determination in essential minerals of the flours of its young shoots enriched with the powders of Moringa (*Moringa oleifera*) leaves and Cowpea (*Vigna unguiculata*) beans, also to evaluate the nutritive contributions from the consumption. For this purpose, the mineral composition of formulations obtained using the central composite design and two industrial infantile flours (ET1 and ET2) were determined.

**Corresponding author: E-mail: mahanromuald@gmail.com;*

The analysis of the macroelements (Ca, P, K, and Na) gave the following contents: Calcium (122.90 ± 2.20 – 355 ± 1.79 mg/100 g), Phosphorus (175.41 ± 0.81 - 481.50 ± 1.75 mg/100 g), potassium (547.95 ± 1.54 - 833.14 ± 2.68 mg/100 g) and sodium (11.14 ± 1.08 - 200.04 ± 2.21 mg / 100 g). The studied flours provided the microelements (Mg, Fe, Cu and Zn) contents following: magnesium (100.11 ± 2.30 - 159.26 ± 2.04 mg/100 g), Iron (5.12 ± 0.08 - 23.20 ± 1.21 mg/100 g), copper (2.84 ± 0.23 - 6.97 ± 0.45 mg/100 g) and zinc (0.63 ± 0.03 - 16.60 ± 0.64 mg/100 g). The average daily quantity of flour consumed by a child from 1 to 2 years in Africa is 250 g. The contributions estimated in minerals of 250 g of EF07 or EE09 flours are more than 3 times higher than those obtained with *B. aethiopum* flour. They contributed to more than 100% of a child for 1 to 2 years minerals needs revealed in this study except for sodium and with more than 50% of the needs of a adult of 70 kg. Similar results are obtained with the reference flours used in this study. The popularization of these composite food formulations could help to ensure the food security of populations, preserve biodiversity and promote the fight against poverty and the advancement of the desert.

Keywords: Enriched flours; B. aethiopum; M. oleifera; V. unguiculata; minerals daily intake.

1. INTRODUCTION

Despite progress in promoting nutrition and health, malnutrition remains widespread, particularly in developing countries, with the highest rate in sub-Saharan Africa [1]. It affects more than 868 million people worldwide, more than 98% of them in developing countries, where at least 225 million children are affected [2,3]. This malnutrition, linked to an overall deficit in energy intakes and to micronutrient deficiencies [4], leads to high infantile mortality, diseases characterized by physical and intellectual retardation and permanent after-effects [5,6].

Deficiencies in some micronutrients such as iron, vitamin A and iodine, which are particularly recognized as been essential to the general health of children and women, can cause blindness, immunodeficiency, mental impairment and even death [7].

Malnutrition, in any form, presents significant threats to human health [8]. Indeed, diets in many African countries are dominated by protein, mineral and vitamin-deficient starchy foods [1].

In Côte d'Ivoire, the prevalence of malnutrition among children under 5 years is 30.0% with 12% severe form and underweight affects 15.0% of children [9].

In addition, mineral elements, essential to life and health are provided by the consumption of the food that contains them, because the organism cannot synthesize them. The mineral elements play a considerable role in all the biochemical exchanges that preside over the perpetuation of life. Thus, they contribute both to the normal metabolism of macronutrients (lipids, carbohydrates and proteins) and to good muscular, cognitive, visual functioning. They also participate in the manufacture of certain hormones and enzymes, help protect against certain toxic substances and regulate organic functions through balancing action. Faced with this situation, one way to remedy this problem concerns the enrichment of food from legumes. Thus, Palmyra whose young shoots are tuberous and edible foods highly valued by the local populations as energetic food resource [10] attracted our attention. The Young Palmyra shoots are often processed into flour for the preparation of porridge or local fufu (food of Côte d'Ivoire), especially during the lean season [11]. Some previous work has described the nutritional composition of this plant material [12,13]. The results of these studies have shown that young shoots of *Borassus aethiopum* are poor in protein and micronutrients like most starchy foods. This nutritional deficit, favourable to the installation of a public health problem, could be corrected by enriching the dishes based on these young shoots of *B. aethiopum* with other local edible vegetable sources, notably cowpeas and *Moringa oleifera*, referring to the recommendations of FAO and WHO [14]. From Cowpea beans, previous attempts reported high quality proteins in significant contents of about 25% [15,13]. As for *Moringa oleifera*, the nutritional reputation concerns mainly leaves that are good sources of minerals and vitamins [16].

The objective of this study is to produce composite flour made from *B. aethiopum* young shoots enriched with *Moringa oleifera* leaves and cowpea seeds that can cover the minerals need of the populations.

2. MATERIALS AND METHODS

2.1 Plant Material

The plant material was the flour processed from Palmyra new shoots tubers, and powders of *Moringa oleifera* leaflets and Cowpea beans. Some industrial infantile flours have been used like references.

2.2 Sampling

The raw material samples were collected between August and December 2015 from three localities, especially Toumodi, Dimbokro, and Didiévi, located in the Centre Region, which are the natural habitat accommodating with Palmyra in Côte d'Ivoire and where large quantities of Cowpea and *Moringa oleifera* are also produced. Three retailers of Palmyra shoot tubers and Cowpea beans were considered per town, and then 30 kg tubers and 10 kg beans were perceived from each retailer, giving total amount of 270 kg Palmyra tubers and 90 kg Cowpea beans. In addition, 50 kg fresh leaflets of *Moringa oleifera* were collected from two sites in each town, 25 kg/site, leading to 150 kg leaves. Once acquired, the samples have been conveyed to the laboratory for analyses. Thus, a pool was constituted by mixing samples by plant species. Finally, 250 kg, 75 kg and 75 kg of respective samples from Palmyra new shoots tubers, Cowpea beans, and *Moringa oleifera* leaves were deducted, sorted, washed meals.

2.3 Processing for Palmyra Flour and Powders from Cowpea and *Moringa oleifera*

Palmyra flour and powders from Cowpea beans and *Moringa oleifera* leaves were processed according to previous reports of Mahan et al. [13]. The Palmyra new shoots tubers were washed, boiled, peeled, carved, rinsed, and then submitted to fermentation into a tank container for 24 h [17]. The resulted fermented tubers pieces were dried at 65℃ into a ventilated oven (Minergy Atie Process, France) for 6 h, and ground using a hammer mill (Forplex).

The *Moringa oleifera* leaves were disinfected for 5 min with chlorinated water (50 mL of 8% sodium hypochlorite in 30 L of water), rinsed, and fermented into a tank for 24 h. Then, fermented leaves were dried at 30℃ for 10-14 days with shade ambient temperature and powdered.

Regarding Cowpea, beans were washed, soaked, drained, and submitted to sprouting at 30℃ during 48 h. The seeds were dried at 40℃ using the previous oven for 96 h, and the resulted malt was sprout out, heated for 15 min in boiling water and submitted to 24 h fermentation into tank. The fermented Cowpea beans were strained, roasted, dried at 50℃ in the oven for 24 h, and ground.

Finally, flour and powders were filtered using sieves with 250 µm diameter and the resulting products were put in polyethylene hermetic bags and kept into dry place till analyses.

2.4 Preparation of Composite Flours

From the flours obtained previously, 15 formulations in different proportions were constituted according to the method used by [18]. Indeed, these authors used a central composite design taking into account 3 variables (quantities of *B. aethiopum*, cowpea and *Moringa oleifera* leaves). Referring to [19], the combination of the 3 variables led to 20 formulations with 8 factorial essays, 6 star essays and 6 essays in the central experimental domain. The 6 essays in the center were reduced to a formulation because they had the same proportions of the ingredients. The different composite flours, the reference flours and their code are presented in the Table 1.

Table 1. Different composite flours, references flours and their code

Flour code	Quantity of flour (%)		
	BAM	VUW	MOL
EF01	72	18	10
EF02	78	14	8
EF03	62.5	28.5	9
EF04	70	23	7
EF05	65	16	19
EF06	71.6	12.8	15.6
EF07	57	26	17
EF08	64.5	21.5	14
EE09	61	24	15
EE010	72	17	11
EE011	75	11	14
EE012	61.4	27.3	11.3
EE013	73	21.6	5.4
EE014	62.8	18.6	18.6
EC015	67.5	20	12.5
ET1		Control 1	
ET1		Control 2	

BAM, Borassus aethiopum Mart., VUW, Vigna unguiculata Walp., MOL, Moringa oleifera Lam

2.5 Samples Mineralization

Samples were mineralized in ashes by incineration at 550℃ using electric muffle furnace. The ashes were obtained after incineration of 5 g flour beforehand carbonized on a Bunsen burner, for 12 h, leading to a white residue [20].

2.6 Mineral Elements Evaluation

The minerals contents of the studied flours wererecovered from ashes using an Energy Dispersive Spectrometer device.

2.6.1 Operating conditions of the energy dispersion spectrometer (EDS)

The energy dispersive spectrophotometer apparatus used for the minerals determination was coupled with a scanning electronic microscope, operating at variable pressure (SEM-FEG Supra 40 Vp Zeiss), and equipped with an X-ray detector (Oxford instruments) bound to a flat shape of the EDS microanalyser (Inca cool dry, without liquid nitrogen). The operative conditions of the EDS-SEM device are:

- Zoom: 10x to 1000000x;
- Resolution: 2 nm;
- Variable voltage: 0.1 KeV à 30 KeV.

The chemical elements were acquired with following parameters: zoom, 50 x; probe diameter, 30 nm to 120 nm; probe energy, 20 KeV and 25 KeV; work distance (WD), 8.5 mm. The chemical composition was explored from 3 different zones, and then the data was transferred to MS Word and Excel softwares for treatment.

2.6.2 Validation test of the minerals determination method

The mineral analysis method has been validated according to standard procedure [21,22], consisting in determination of the linearity, repeatability, reproducibility, extraction yields, and detection and quantification limits. The linearity of 10 mineral elements was valued using 5 standard points between 25% and 125% (25%, 50%, 75%, 100%, and 125%). The repeatability and reproducibility tests were achieved with standard of the different minerals at the content of 25%. A percentage of 5% was added on each mineral's standard content for determining the yield of mineral extraction. Ten separate tests were performed for the proportions added.

2.7 Contributions Estimated in Essential Elements at the Consumer

The contributions in mineral elements have been estimated according to the method of the Codex Alimentarius that takes into account the concentrations in minerals recovered in the food and the daily consumption of a child of 1 to 2 years of this food. The contribution of this food in daily requirement has been calculated also from the values of daily recommended intakes [23].

$$\text{Estimated Daily Intake (EDI)} = C \times Q$$
$$\text{Contribution (\%)} = (\text{EDI} \times 100)/\text{DRI}$$

With: C, mineral concentration measured; Q: food daily consumption; DRI: Daily Recommended Intake

2.8 Statistical Analysis

The data were recorded with Excel file and statistically treated with Statistical Program for Social Sciences (SPSS 22.0 for Windows). The statistical test consisted in a one-way analysis of variance (ANOVA) with the type of meal assessed basis. From each parameter, means were compared using Student Newman Keuls post-hoc test at 5% significance level. In addition, Multivariate Statistical Analysis (MSA) was performed through Principal Components Analysis (PCA) and Hierarchical Ascending Clustering using STATISTICA software (version 7.1) for structuring correlation between the samples studied and their minerals traits.

3. RESULTS

3.1 Validation Parameters for Quantification of Minerals Using EDS

The data from validation assays are reported in Table 2. The determination coefficient (R^2) recovered from the standard lines are included between 0.99 and 1. The minerals limits of detection vary from 104 µg/kg to 581 µg/kg and their minimal values quantified are between 146 µg/kg and 796 µg/kg. The coefficients of variation (CV) from 10 repeatability tests oscillate between 1.1% and 1.8%; while 15 reproducibility tests result in CV from 2.3% to 4.7%. About the minerals added, the extraction yields run from 97.3% to 99.5%, revealing minerals extraction defaults between 0.5% and 2.7%.

3.2 Mineral Composition of Flour

The mineral contents of the different flours studied are presented in Table 3. The results indicate that the calcium (Ca) contents range from 122.90 ± 2.20 mg / 100 g (EE13) to 355 ± 1.79 mg/100 g (ET2). Iron (Fe) contents ranged from 5.12 ± 0.08 mg/100 g (EE13) to 23.20 ± 1.21 mg mg/100 g (ET1). The results show that calcium and iron contents of the composite flours EF05, EF07 and EE14 are respectively higher than those of commercial flours ET1 and ET2. The composite flours EF06, EF08, EE09, EE11, EE12 and EC15 had statistically identical iron contents (p>0.001) compared to commercial flour ET2.

The magnesium (Mg) and copper (Cu) contents of the composite flours are ranged respectively between 100.11 ± 2.30 mg/100 g (EF02) and 159.26 ± 2.04 mg/100 g (EF07), between 2.84 ± 0.23 mg/100 g (EE13) and 6.97 ± 0.45 mg/100 g (EF05). These levels are significantly higher than those of commercial flours ET1 and ET2(P <0.001).

Composite flours contain levels of zinc (Zn) (0.63 ± 0.03 to 2.21 ± 0.15 mg/100 g), sodium (Na) (11.14 ± 1.08 to 22.17 ± 0.71 mg/100 g) and phosphorus (P) (175.41 ± 0.81 to 260.12 ± 1.60 mg/100 g) were statistically low (P <0.001) for values resulting from commercial flours ET1 and ET2. However, EF07 composite flour had the highest values (22.17 ± 0.71 and 260.12 ± 1.60 mg/100 g), respectively, of Na and P.

Concerning potassium (K), the majority mineral, the grades vary from 547.95 ± 1.54 (EF02) to 833.14 ± 2.68 mg/100 g (EF07). The composite flours EF03, EF05, EF07, EF08, EE09, EE12, EE14 and EC15 provide the highest values compared to commercial flours ET1 (610 ± 3.15 mg/100 g) and ET2 (679.02 ± 1.63 mg/100 g) while the composite flours EF01, EF04, EF06, EE10 and EE11 contain the contents between those of ET1 and ET2.

3.3 Grouping of Samples According to Minerals

Principal component analysis (PCA) was carried out by considering components F1 and F2 (Table 4), which have an eigenvalue greater than 1, according to the Kaïser statistical rule. Emphasized groupings of the PCA were then clarified by the hierarchical ascending classification (CAH) using the Unweighted Pair Group Method with Arithmetic Means (UPGMA).

3.3.1 Principal component analysis (PCA)

Fig. 1.A shows the circle of correlations of the factorial axes F1 and F2, which express 88.87% of the total variability of the studied parameters. The component F1 with an eigenvalue of 4.71, expresses 58.92% of the variance. It is predominantly established by positive correlations with the phosphorus, sodium, iron and zinc contents and a negative correlation with the copper content. The component F2, with its own value 2.4, expresses 29.95% of the variance and is mainly formed by the calcium and potassium contents with negative correlations (Table 4).

The projections of the characteristics and of the samples in the plane formed by the components F1 and F2 highlight two groups of flours. Group 1 consisted essentially of commercial flours ET1 and ET2. Those are characterized by contents of iron, calcium, zinc, sodium and of phosphorus higher compared to the values resulting from the produced composite flours. Group 2 contains the composite samples of flours. They provide higher levels of magnesium, potassium and copper than those of control flours (Fig. 1.B).

3.3.2 Hierarchical ascending classification

Hierarchical classification also reveals two classes of samples of flours, with the Euclidean distance from aggregation of 182.

Class 1 represents commercial flours ET1 and ET2. Samples in this class are distinguished by higher levels of iron, calcium, zinc, sodium and phosphorus than other analyzed samples. Class 2 contains samples composite of flours. Class 2 samples have the highest magnesium, potassium and copper values (Fig. 2).

3.4 Estimated Intakes and Contribution of Essential Mineral Elements in Children or Adults

Sensory evaluation tests of the slurries prepared from the composite flours studied made it possible to retain the flours EF07 and EE09 having exhibited the most interesting sensory characteristics. The quantities of mineral elements contributed by these two composite flours (EF07 and EE09), reference flours (ET1 and ET2) and *B. aethiopum* flour and their contribution to the estimated total input are evaluated.

Table 2. Data from validation parameters for evaluation of minerals contents using energy diffusion spectrometer (EDS)

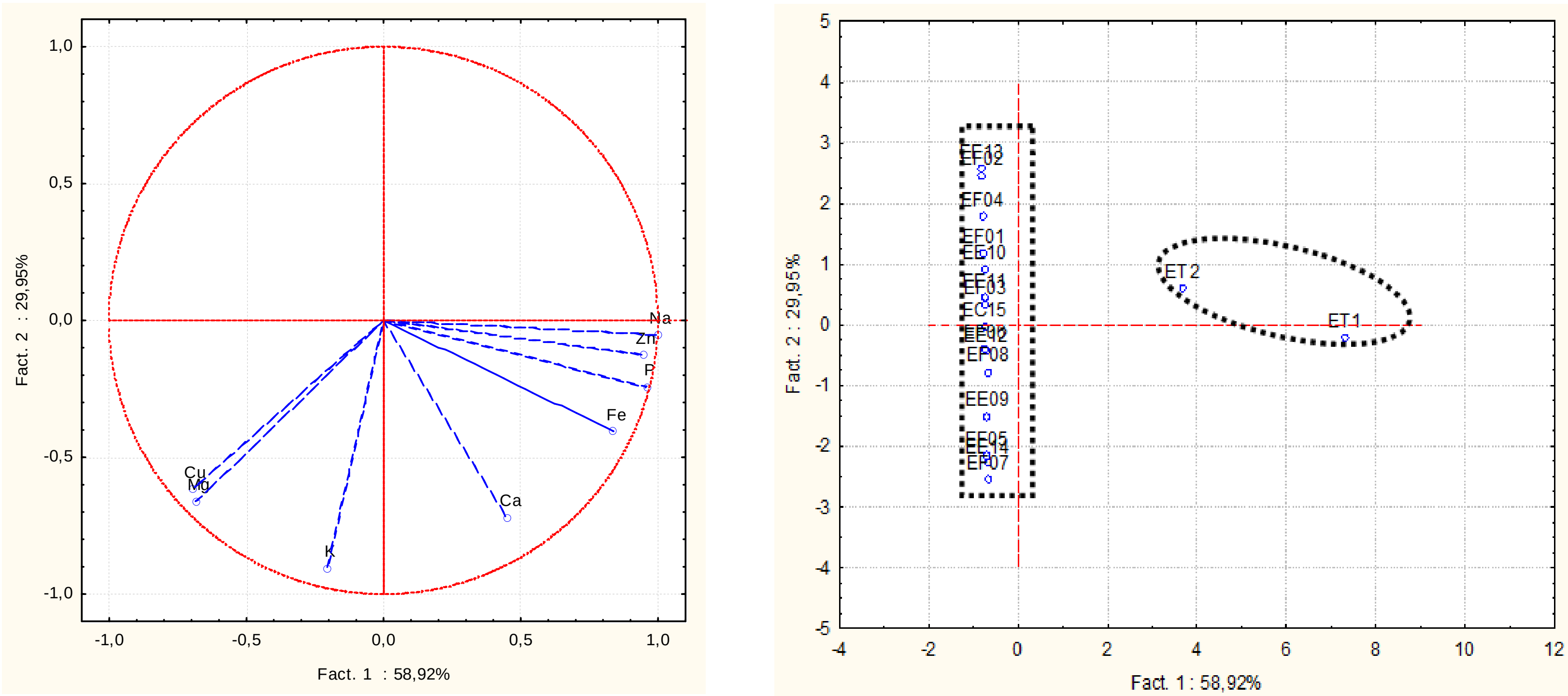
Validation parameters		**Potassium**	**Magnesium**	**Phosphorus**	**Calcium**	**Sodium**	**Iron**	**Copper**	**Zinc**
Linearity	**ESL**	Y=3821x+3838	Y=1452x+237	Y=2667x+1742	Y=6581x+5287	Y=2083x+147	Y=2285x-88	Y=1953x+6951	Y=4365x-523
	CD (R^2)	1	0,99	0,99	1	0,99	0,99	0,99	0,99
CV repeat (%)		1.3±0.04	1.1±0.21	1.4±0.11	1.5±0.43	1.2±0.05	1.4±0.07	1.8±0.95	1.3±0.51
CV reprod (%)		4.7±0.32	3.1±1.44	3.7±1.22	2.3±0.93	3.4±0.48	3.6±0.01	2.5±0.3	3.2±0.96
EYAM (%)		98.4±1.51	97.9±0.68	99.4±0.66	97.3±0.84	98.8±0.33	99.5±0.17	98.8±0.43	98.3±0.03
LOD (µg/kg)		581±0.04	426±0.11	334±0.21	514±0.15	261±0.74	107±0.32	104±0.05	281±0.58
LOQ (µg/kg)		796±0.09	635±0.19	467±0.88	704±0.47	365±0.07	149±0.55	146±0.63	396±0.29

ESL, equation of standard lines; CD, coefficient of determination; CV repeat, coefficient of variation from repeatability test; CV reprod, coefficient of variation from reproducibility test; EYAM, extraction yield from added minerals; LOD, limit of detection; LOQ, limit of quantification

Table 3. Contents in minerals of the studied flours

Samples	**Microelements**				**Microelements**			
	Calcium	**Phosphorus**	**Potassium**	**Sodium**	**Magnesium**	**Iron**	**Copper**	**Zinc**
EF01	177.25 ± 1.81^{l}	198.95 ± 1.21^{j}	625.45 ± 1.91^{l}	14.65 ± 0.61^{fg}	115.43 ± 2.16^{g}	6.78 ± 0.33^{f}	4.25 ± 0.16^{ef}	1.17 ± 0.08^{hij}
EF02	146.88 ± 1.78^{m}	175.41 ± 0.81^{l}	547.95 ± 1.54^{p}	12.45 ± 0.48^{gh}	100.11 ± 2.30^{i}	5.70 ± 0.35^{g}	3.73 ± 0.23^{fg}	0.94 ± 0.06^{ijk}
EF03	177.17 ± 1.67^{l}	231.39 ± 2.37^{f}	719.41 ± 1.26^{g}	15.04 ± 0.77^{fg}	128.21 ± 1.85^{e}	7.05 ± 0.06^{ef}	3.79 ± 0.32^{fg}	1.05 ± 0.03^{hij}
EF04	145.00 ± 1.27^{m}	202.55 ± 1.30^{i}	625.94 ± 2.07^{l}	12.68 ± 0.84^{gh}	110.44 ± 1.42^{h}	5.87 ± 0.42^{g}	3.29 ± 0.25^{gh}	0.81 ± 0.08^{jk}
EF05	290.37 ± 1.83^{b}	234.14 ± 1.64^{f}	762.01 ± 2.25^{d}	22.08 ± 1.29^{c}	151.68 ± 3.45^{b}	10.35 ± 0.20^{b}	6.97 ± 0.45^{a}	2.21 ± 0.15^{c}
EF06	242.44 ± 2.28^{f}	206.86 ± 1.55^{h}	668.41 ± 1.21^{j}	18.79 ± 1.10^{de}	131.52 ± 1.48^{e}	8.75 ± 0.31^{cd}	5.94 ± 0.42^{c}	1.82 ± 0.13^{cde}
EF07	276.27 ± 2.46^{c}	260.12 ± 1.60^{c}	833.14 ± 2.68^{a}	22.17 ± 0.71^{c}	159.26 ± 2.04^{a}	10.16 ± 0.05^{b}	6.17 ± 0.46^{bc}	2.06 ± 0.16^{cd}
EF08	232.53 ± 2.63^{g}	233.13 ± 2.24^{f}	732.74 ± 1.21^{f}	18.47 ± 1.07^{de}	135.72 ± 1.68^{d}	8.65 ± 0.11^{cd}	5.37 ± 0.33^{cd}	1.64 ± 0.04^{defg}
EE09	248.32 ± 1.33^{e}	243.65 ± 1.88^{d}	776.83 ± 2.21^{c}	20.62 ± 1.31^{cd}	147.20 ± 2.23^{c}	9.23 ± 0.08^{c}	5.62 ± 0.46^{cd}	1.75 ± 0.11^{def}
EE10	188.80 ± 2.45^{k}	200.15 ± 1.94^{ij}	632.35 ± 0.79^{k}	15.36 ± 0.93^{f}	118.20 ± 2.86^{fg}	7.20 ± 0.17^{ef}	4.56 ± 0.37^{ef}	1.29 ± 0.10^{ghi}
EE11	219.84 ± 2.22^{h}	192.98 ± 1.73^{k}	621.24 ± 1.63^{m}	17.21 ± 1.31^{ef}	121.55 ± 2.20^{f}	7.98 ± 0.63^{de}	5.53 ± 0.42^{cd}	1.65 ± 0.06^{defg}
EE12	205.08 ± 1.46^{j}	237.95 ± 2.26^{e}	746.99 ± 1.36^{e}	16.86 ± 0.87^{ef}	136.35 ± 3.08^{d}	7.92 ± 0.25^{de}	4.48 ± 0.27^{ef}	1.33 ± 0.08^{fghi}
EE13	122.90 ± 2.20^{n}	190.09 ± 1.32^{k}	583.02 ± 2.86^{o}	11.14 ± 1.08^{h}	101.12 ± 1.94^{i}	5.12 ± 0.08^{g}	2.84 ± 0.23^{h}	0.63 ± 0.03^{k}
EE14	287.75 ± 1.80^{b}	241.46 ± 1.65^{d}	782.60 ± 3.18^{b}	22.05 ± 1.87^{c}	154.18 ± 3.10^{b}	10.36 ± 0.32^{b}	6.76 ± 0.63^{ab}	2.17 ± 0.02^{c}
EC15	211.61 ± 2.14^{i}	217.77 ± 2.09^{g}	690.51 ± 1.86^{h}	17.01 ± 0.70^{ef}	129.68 ± 1.80^{e}	7.94 ± 0.69^{de}	4.95 ± 0.64^{de}	1.46 ± 0.24^{efgh}
ET1	266.01 ± 1.70^{d}	481.50 ± 1.75^{a}	610.00 ± 3.15^{n}	200.04 ± 2.21^{a}	75.17 ± 0.40^{j}	23.20 ± 1.21^{a}	1.14 ± 0.06^{i}	16.60 ± 0.6^{4a}
ET2	355.00 ± 1.79^{a}	378.01 ± 1.95^{b}	679.02 ± 1.63^{i}	108.00 ± 1.58^{b}	52.14 ± 1.38^{k}	9.00 ± 0.50^{cd}	0.39 ± 0.01^{j}	4.50 ± 0.15^{b}
F	2883.64	5575.34	4563.35	4976.59	490.23	246.16	70.27	1176.74
P	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**

Values are expressed in mg of mineral for 100 g of dry matter. From the same column, values differ statistically at P=5% according to the lowercase letter. F, value of the Ficher statistical test of ANOVA; P-value, value of the ANOVA probability test



(A) **(B)**

Fig. 1. Correlations drawn between the F1-F2 factorial design of the PCA and the mineral parameters (A) and samples (B) of the studied flours

Estimated mineral intakes of EF07 and EE09 flours are comparable to the majority of those of reference flours and more than 3 times higher than those of the flour of young shoots of *B. aethiopum*. The EF07 and EE09 flours recorded the daily intakes of Ca, P, K, Na, Mg, Fe, Cu and Zn, respectively of (690.68 and 620.8 mg/day) (650.3 and 609.13 mg/day), (2082.85 and 1942.08 mg/day), (55.43 and 51.55 mg/day), (398.15 and 368 mg/ day), (25.4 and 23.08 mg (15.43 and 14.05 mg/day), and (5.15 and 4.38 mg/day).

The daily intakes of Calcium, phosphorus, potassium, sodium, magnesium, iron, copper and Zinc of reference flours ET1 and ET2 are respective 665.03 and 887.5 mg/day, 1203.75 and 945.03 mg/day, 1525 and 1697.55 mg/ day, 500.1 and 270 mg/day, 187.93 and 130.35 mg/day, 58 and 22.5 mg/day, 2.85 and 0.98 mg/day and 41.5 and 11.25 mg/day. The flour of young shoots of *B. aethiopum* yields the lowest values in Ca (69.63 mg/day), P (220.83 mg/day), K (647.35 mg/day), Na (9.8 mg/day), Mg (105.15 mg/day), Fe (3.8 mg/day), Cu (4.03 mg/day) and in Zn (< 0.07 mg/day) (Table 5).

Contributions of essential mineral inputs of composite flours (EF07 and EE09) and reference flours (ET1 and ET2) are higher than the recommendations of the Joint FAO / WHO and WHO Joint Committee for 1- to 2-year-olds except sodium which provides a contribution of 11.09% (EF07), 10.31% (EE09) and 54% (ET1).

The consumption of 250 g of flour intended for the children from 1 to 2 years by the adult of 70 kg gives also better results. EF07 flour also covers the daily requirements for K, Mg, Fe and Cu for an adult of 70 kg and contributes to 86.34%, 92.9%, 2.22% and 51.5% of the requirements Ca, P, Na and Zn respectively. The flour EE09 provides the recommended daily amounts of iron and copper for the adult 70 kg and contributes to 77.6%, 87.02%, 97.1%, 2.06%, 98.13% and 43.8% of the recommended daily intake of Ca, P, K, Na, Mg and Zn respectively. Similarly, the reference flours used cover the P, Fe, Cu and Zn requirements (Table 6).

Table 4. Eigenvalue matrix and correlations of the mineral parameters of flours studied with components F1 and F2 of the principal component analysis

Components	F1	F2
Eigenvalue	4.71	2.4
Validity expressed (%)	58.92	29.95
Cumulative validity expressed (%)	58.92	88.87
Calcium	0.45	**-0.72**
Phosphorous	**0.96**	-0.24
Potassium	-0.21	**-0.9**
Sodium	**1**	-0.05
Copper	**-0.7**	-0.62
Iron	**0.84**	.-0.41
Magnésium	-0.68	-0.66
Zinc	**0.95**	-0.13

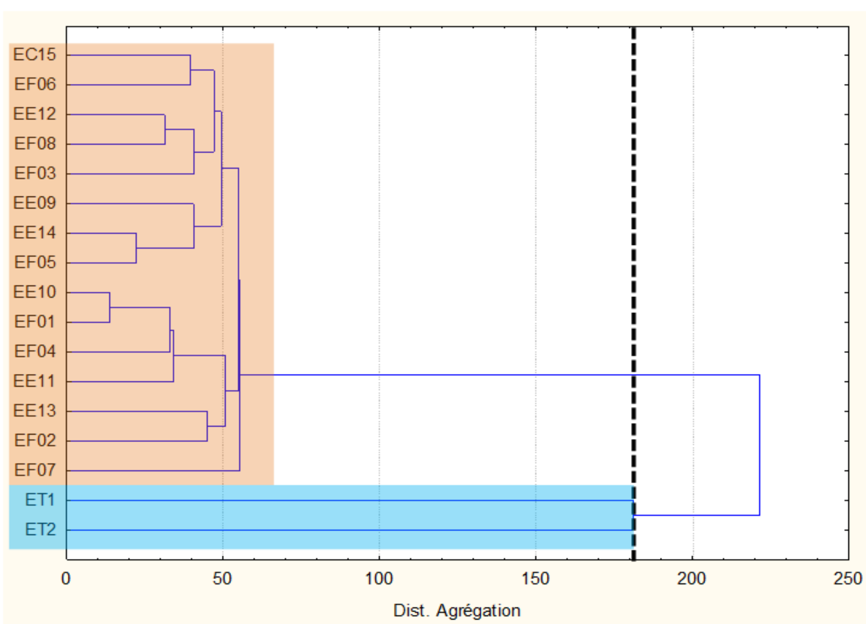


Fig. 2. Hierarchical classification of meals studied according to mineral characteristics

Table 5. Daily intakes of minerals resulting from the consumption of 250 g of flour

Flour	Estimated intake in mg/day in 1- to 2-year-old or an adult of 70 kg							
	Macroelements					Microelements		
	Calcium	**Phosphorus**	**Potassium**	**Sodium**	**Magnesium**	**Iron**	**Copper**	**Zinc**
BAMF	69.63	220.83	647.35	9.8	105.15	3.8	4.03	< 0.07
EF07	690.68	650.3	2082.85	55.43	398.15	25.4	15.43	5.15
EE09	620.8	609.13	1942.08	51.55	368	23.08	14.05	4.38
ET1	665.03	1203.75	1525	500.1	187.93	58	2.85	41.5
ET2	887.5	945.03	1697.55	270	130.35	22.5	0.98	11.25

BAMF: B. aethiopumMart fermented;EF07: Flour composed of 57% B. aethiopum, 26% cowpea and 17% M. oleifera; EE09: Flour composed of 61% B. aethiopum, 24% cowpea and 15% M.oleifera; ET1: reference flour 1; ET2: reference flour 2.

Table 6. Daily minerals recommended intakes and contribution of flour minerals

Flour	Macroelements										Microelements					
	Calcium		Phosphorus		Potassium		Sodium		Magnesium		Iron		Copper		Zinc	
	DRI	**Contr**	**DRI**	**Contr**	**DRI**	**Contr**	**DRI**	**Contr**	**DRI**	**Contr**	**DRI**	**Contr**	**DRI**	**Contr**	**DRI**	**Contr**
	In the 1- to 2-year-old															
EF07		138.14		180.64		208.29		11.09		497.69		479.25		2204.3		125.61
EE09	**500**	124.16	**360**	169.2	**1000**	194.21	**500**	10.31	**80**	460	**5.3**	435.47	**0.7**	2007.1	**4.1**	106.83
ET1		133.01		334.38		152.5		100.02		234.91		1094.3		407.14		1012.2
ET2		177.5		262.51		169.76		54		163.19		424.53		140		274.39
	In an adult of 70 kg															
EF07		86.34		92.9		104.14		2.22		106.17		181.43		1543		51.5
EE09	**800**	77.6	**700**	87.02	**2000**	97.1	**2500**	2.06	**375**	98.13	**14**	164.86	**1**	1405	**10**	43.8
ET1		83.13		171.96		76.25		20		50.11		414.29		285		415
ET2		110.94		135		84.88		10.8		34.76		160.71		98		112.5

Sources (DRI): FAO/WHO (2002), WHO (2007)

EF07: Flour composed of 57% B. aethiopum, 26% cowpea and 17% M. oleifera; EE09: Flour composed of 61% B. aethiopum, 24% cowpea and 15% M.oleifera; ET1: reference flour 1; ET2: reference flour 2; DRI, daily recommended intake (mg/day), contr, contribution (%)

4. DISCUSSION

The R^2 determination coefficients got from the calibrations tests were close to 1, forecasting a quasi-linear estimation of the mineral nutrients according to their concentration from in the meals. Also, the lower coefficients of variation (<5%) resulting from reproducibility and repeatability translate quite stability of the EDS method used, which is as fitted as the full amount of each mineral nutrient is revealed, as shown by the weak extraction defaults below 2.7% from the added minerals. Thus, these characteristics highlight the reliability and precision of the outcomes in the minerals contents determination using the EDS method.

Results indicate that the enrichment significantly ($p < 0.001$) contributed to increase the mineral content of young shoots of *B. aethiopum* flour. This could be explained by the fact that *Moringa oleifera* used for fortification would have high micronutrient levels. Also, cowpea would contribute to improving the mineral content of young shoots of *B. aethiopum* flour. These results are similar to those of other studies which presents *Moringa oleifera* and Cowpea as vegetable and natural reserves of micronutrients and proteins [24,25,16,15].

Thus, this supplementation permitted to obtain composite flours EF05, EF07 and EE14 whose iron contents are higher than those of the control flour ET2. Iron is a trace metal essential in a variety of vital functions, including oxygen transport, DNA synthesis, metabolic energy, and cellular respiration [26,27]. The enrichment of *B. aethiopum* flour also permitted to obtain composite flours EF03, EF05, EF07, EF08, EE09, EE12, EE14 and EC15,of witch potassium contents were higher than those of the commercial flours ET1 and ET2.

Also, the flours obtained have magnesium contents higher than those of the commercial flours ET1 and ET2.Magnesium is an essential component of the body, required in relatively large quantities compared to other minerals because it plays a crucial role in the structure of the skeleton and muscles [28].

The composite flours EF05, EF07 and EE14 are a good source of calcium compared to flour reference ET1.Calcium is required for normal growth, strong muscles and skeletal development [29]. However, the low sodium content of the plant material used for the formulation of composite flours [30] may explain their low sodium content.

The zinc content of the composite flours obtained is insufficient. Yet, Zinc is essential for growth and development, testicular maturation, neurological function, wound healing and immunocompetence [31]. Also, phosphorus levels are lower than those of the used commercial infantile flours. The low phosphorus levels of composite flours based on young shoots of the *B. aethiopum* can be explained by losses due to diffusion in the soaking and cooking water.

In order to fill this deficiency in sodium, zinc and phosphorus, it would be advisable, on the one hand, to soak the chips of young shoots of *B. aethiopum* and cowpea seeds in water containing iodized salt, and on the other hand, to add to the consumption of these flours an additional supplement such as fruits, honey or milk. The flours studied contain a good copper content with beneficial effects on the immune system [28].

The average daily quantity of flour consumed by a child aged 1 to 2 years in Africa is 250 g [32]. During the intake of the same quantity, the estimated daily intakes in the 1-to-2-year-old or the 70-kg adult were much higher in composite flours (EF07 and EE09) and in control flours compared to flour of *B. aethiopum*. This distribution shows the importance of the fortification of the flour of young shoots of *B. aethiopum* to the powders of *Moringa oleifera* leaves and cowpea beans. Indeed, the incorporation of the powders of *Moringa oleifera* leaves and cowpea beans into the flour of young shoots of *B. aethiopum* significantly contributed to increase the mineral contents. Composite flours EF07 and EE09 have mineral characteristics comparable to industrial infantile flours. Besides, they cover the daily recommended intake in mineral at the children of 1 to 2 years by WHO [33]. Composite flours EF07 and EE09 can meet the nutritional needs of populations, especially children. Healthy essential mineral intakes could help meet the ever-increasing needs of children aged 1 to 2 years and be beneficial in combating malnutrition.

5. CONCLUSION

This study showed that the enrichment of the flour of young shoots of *B. aethiopum* to the powders of *Moringa oleifera* leaves and cowpea seeds is a considerable improvement of the nutritive characteristics of these flours. The majority of the formulations obtained have very appreciable contents of minerals.

Among these composite flours, the EF07 and EE09 flours provided a very valuable daily intake of minerals, thus contributing efficiently to the needs of consumers.

The intake estimate showed that flours EF07 and EE09 cover the mineral requirements of children aged 1 to 2 years and a larger share of daily intake in adults. Composite flours EF07 and EE09 with mineral characteristics comparable to industrial infantile flours are an asset in the fight against malnutrition which threatens populations during the lean season. The production of these composite flours can promote the cultivation of these plant species, protect biodiversity and generate significant economic returns.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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