Journal of Advances in Biology & Biotechnology
13(1): 1-12, 2017; Article no.JABB.32848
ISSN: 2394-1081

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Vitamin Contents and Nutritive Contribution of Flours of Palmyra New Shoots Enriched with *Moringa oleifera* Leaves and Cowpea (*Vigna unguiculata*) Powders

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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 KNY and AYO. Authors DMV, KNY 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.

Article Information

DOI: 10.9734/JABB/2017/32848
Editor(s):
(1) Fernando José Cebola Lidon, Universidade Nova de Lisboa, Campus da Caparica, Portugal.
Reviewers:
(1) Muhammad Nadeem, University of Sargodha, Sargodha, Punjab, Pakistan.
(2) E. Ojokoh, Akanu Ibiam Federal Polytechnic, Nigeria.
(3) Samson A. Oyeyinka, University of Ilorin, Nigeira.
Complete Peer review History: http://www.sciencedomain.org/review-history/19203

Original Research Article

Received 18th March 2017
Accepted 16th May 2017
Published 26th May 2017

ABSTRACT

The aim of this study is to contribute to a better valorization of *Borassus aethiopum* by the content determination in vitamin of new shoots of Palmyra-based enriched flour, also to evaluate the nutritive contributions from the consumption.
Fifteen composite flours gotten from flours of *B. aethiopum*, *M. oleifera* leaves and *V. unguiculata*

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beans powders previously treated, *Borassus aethiopum* new shoots flour, and two commercial control flours (ET1 and ET2) were analysed. HPLC techniques were used for the separation and quantification of β-carotene and vitamin E and the water-soluble vitamins (vitamins B1, B2, B6 and B9). Vitamin C contained in analyzed samples was determined by titration. Also, estimated daily intake has been evaluated for 1- to 2-year-old.
The water-soluble vitamins analysis gave for 100 grams of dry matter the following contents: vitamin C (23.58 - 60.03 mg), vitamin B1 (0.21 - 1.00 mg), vitamin B2 (0.29 - 1.09 mg), vitamin B6 (0.0 - 1.00 mg) and vitamin B9 (0.22 - 0.41 mg). The studied flours provided the fat-soluble vitamins contents following: β-carotene (111.67 - 960 ER/100 g) and vitamin E (0.00 - 15.95 mg/100 g). The average daily amount of flour consumed by a 1- to 2-year-old in Africa is 250 g. The contributions estimated in vitamins a 250-gram flours EF07 or EE09 were similar to those provided with the references 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; vitamins daily contribution.

1. INTRODUCTION

Malnutrition due to nutritionally inadequate diets is a major concern in Côte d'Ivoire and many other developing countries. 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 852 million people worldwide, more than 95% of them in developing countries, where at least 250 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 40.6% with 15.7% severe form and underweight affects 20.2% of children, with 4.3 % severe form [9]. Furthermore, the body needs vitamins, but it is unable to synthesize them or to make sufficient quantities with the exception of vitamins K and D. They must be brought to the body through food. Vitamins are regulators of synthetic and degradation processes and constitute the structural elements of coenzymes, hormones and other substances. They play a role in the growth, repair and proper functioning of the organism and have above all a catalytic function. Faced with this situation, one way to remedy this problem concerns the enrichment of food from legumes. Thus, *Borassus aethiopum* whose young shoots are tuberous and edible foods highly valued by the local populations as energetic food resource [10] attracted our attention. Palmyra young shoots are often processed into flour for the preparation of porridge or local fufu (food found in 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 is a threat to the public which could cause health problem. The dishes enrichment 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] would contribute to solve the problem. 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 [16,17].

The objective of this study is to produce composite flour made from *B. aethiopum* young shoots enriched with high-vitamins *Moringa* *oleifera* leaves and *Vigna unguiculata* beans needed by the population.

2. MATERIALS AND METHODS

2.1 Plant Materials

The plant material was the flour processed from Palmyra new shoots tubers, and powders of *Moringa oleifera* leaves and *Vigna unguiculata* seeds. Some industrial infantile flours were used as reference during the study.

2.2 Sampling

The raw material samples were collected between August and December 2015 from three localities, namely Toumodi, Dimbokro, and Didiévi, located in the Centre Region, which are the natural habitat accommodating Palmyra in Cote d'Ivoire and where large quantities of Cowpea and Moringa 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 purchased from the retailer, giving total amount of 270 kg Palmyra tubers and 90 kg Cowpea beans. In addition, 50 kg fresh leaves of Moringa were collected from two sites in each town, 25 kg/site, leading to 150 kg leaves. Once acquired the samples were taken to the lab 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 leaves were deducted, sorted, washed meals.

2.3 Processing of Palmyra Flour and Powders from Cowpea and Moringa

Palmyra flour and powders from Cowpea beans and Moringa 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 to fermentation with, allowed to ferment inside a tank for 24 hr. [18]. The fermented tubers pieces were dried at 65°C in a ventilated oven (Minergy Atie Process, France) for 6 hr, and ground using a hammer mill (Forplex). The moringa leaves were disinfected for 5 min with chlorinated water (50 mL of 8% sodium hypochlorite in 30 L of water), rinsed, and fermented inside a tank for 24 hr. Then, fermented leaves were dried at 30°C for 10-14 days with shade ambient temperature and powdered. Regarding Cowpea, beans were washed, soaked, drained, and submitted to sprouting at 30°C during 48hr. The seeds were dried at 40°C using the oven for 96hr, and the resulted malt was sprout out, heated for 15 min in boiling water and submitted to 24 hr fermentation inside a tank. The fermented Cowpea beans were strained, roasted, dried at 50°C in the oven for 24 hr, 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 dry place till analyses.

2.4 Preparation of Composite Flours

From the flours obtained, 15 formulations in different proportions were constituted according to the method used by Mahan et al. [19]. Indeed, these authors used a central composite design taking into account 3 variables (quantities of *B. aethiopum*, cowpea and *Moringa oleifera*). Referring to Feinberg [20], 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
EE10	72	17	11
EE11	75	11	14
EE12	61.4	27.3	11.3
EE13	73	21.6	5.4
EE14	62.8	18.6	18.6
EC15	67.5	20	12.5
ET1		Control 1	
ET1		Control 2	

BAM, Borassus aethiopum Mart.; VUW, Vigna unguiculata Walp ; MOL, Moringa oleifera Lam; EF, factorial essay; EE, star essay; EC, essay at the centre ; ET1, sample control 1; ET2, sample control 2

2.5 Determination of Vitamin Content

Vitamin C contained in analyzed samples was determined by titration using the method described by Pongracz et al. [21]. About 10 g of sample were soaked for 10 min in 40 mL metaphosphoric acid-acetic acid (2%, w/v). The mixture was centrifuged at 3000 rpm for 20 min and the supernatant obtained was diluted and adjusted with 50 mL of bi-distilled water. Ten (10) mL of this mixture was titrated to the end point with dichlorophenol-indophenol (DCPIP) 0.5 g/L. A precalibration with ascorbic acid 0.5 g/L was used to determine the value of the vitamin C contained in samples.

On the other hand, the concentrations of water-soluble vitamins of Group B and fat-soluble were determined using a high performance liquid chromatographic system (HPLC, mark Water Alliance). This system included a Waters pump, an automatic injector, a UV / PDA detector and a Servotrace recorder. The operating conditions were adapted to the type of required vitamins.

Table 2. Concentration of injected samples and wavelengths

Vitamins	Concentration range (µg/ml)	Wavelengths (nm)
Vitamin B_1	0.1 to 3.5	270
Vitamin B_2	0.1 to 7	265
Vitamin B_6	0,5 to 12	257
Vitamin B_9	0.5 to 5	280
β-carotène	0.2 to 4.5	445
Vitamin E	0.2 to 5.5	295

Two grams of flour samples were extracted vigorously with an excess of n-hexane (5 times the volume) and centrifuged to 3000 rpm during 5 min. The organic solvent was aspirated and saved. The residue was reextracted with the same solvent and the same steps were repeated until the extract was almost colorless. The total volume of the extract was recorded and an aliquot was injected in the HPLC system. Fat soluble vitamins were separated on a column Kromasil C18 of 30 X 4 mm (CIL CLUZEAV) in stainless steel. The mobile phase was a mixture of acetonitrile of HPLC grade and well furnished by MERCK (Germany). The column temperature was 30 ° C, the elution length was 35 min and the flow rate was 1.2 mL / min. Water soluble vitamins were separated on a Zorbax column to silica support post grafted in C18 (150 mm X 4.6 mm) with particles of 3 mm. The mobile phase was a mixture of ammonium acetate and methanol, of grade HPLC and furnished by MERCK (Germany). The flow rate was programmed to 2 mL / min on a length of 20 min. Standard β-carotene and vitamin E were purchased from Fluka Chemie (Switzerland), while water soluble vitamins were purchased from Sigma-Aldrich (UK). Table 2 present the concentrations of the standard vitamins used for injection in the HPLC system.

2.6 Evaluation of the Nutritive Contribution

Vitamin supply have been estimated according to the method of the Codex Alimentarius that takes into account the concentrations in vitamins recovered in the food and the daily consumption of a 1- to 2-year-old of this food [22].

$$\text{Estimated Daily Intake (EDI)} = C \times Q$$

With: C, Vitamin concentration measured; Q, food daily consumption.

2.7 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 of a one way analysis of variance (ANOVA) with the type of meal assessed. From each parameter, means were compared using Student Newman Keuls post-hoc test at 5% significance level. The software STATISTICA (STATISTICA version 7.1) used for an analysis in principal components (ACP) and an Ascending hierarchical clustering (CAH) in order to structure variability between the samples and the contents of vitamins.

3. RESULTS

3.1 Vitamin Composition of the Flours

The results show that the studied flours contain the water-soluble vitamins (vitamins C, B1, B2, B6 and B9) and fat-soluble vitamins (β-carotene and vitamin E) (Table 3).

The vitamins C and B1 contents of composite flours are statistically identical ($p < 0.001$). The vitamins B2, B6 and B9 contents of composite flours are highly variable. The vitamins B2 and B6 contents varied from 0.29 (EE13) to 0.65 mg/100 g (EF05) and from 0.11 (EE13) to 0.38 mg/100 g (EF05 and EE14) respectively. Those of vitamin B9 ranged between 0.22 (EF02) and 0.41 mg/100 g (EF08).

Composite flours of Palmyra new shoots have the lowest values of vitamins C, B1 and B2 compared to those of commercial infantile flours ET1 (60.03; 1 and 1 mg/100 g respectively) and ET2 (46.20, 0.70 and 1.09 mg/100 g, respectively). On the other hand, vitamin B9 contents of composite flours are higher than commercial flour ET2 (0.07 mg/100 g) and the majority are statistically identical ($p < 0.001$) to commercial infantile flour ET1 (0.29 mg/100 g) (Table 3). As for vitamin B6, EF05 and EE14 (0.38 mg / 100 g) composite flours provide statistically identical contents ($p < 0.001$) comparatively to commercial flour ET2 (0.40 mg/100 g). Palmyra flour didn't contain the vitamin B6 and provided the lowest contents in vitamins B2 (0.13 mg/100 g), and B9 (0.16 mg/100 g) compared to the other studied flours.

Regarding fat-soluble vitamins, their contents in composite flours are more appreciable. Thus, all enriched flours contain the highest levels of β-carotene (703.33 - 960 RE/100 g) compared to Palmyra flour (648.33 RE/100 g), commercial flours ET1 (445 RE/100 g) and ET2 (111.67 RE/100 g). The obtained composite flours also contain the highest contents of vitamin E (4.54 - 15.95 mg/100 g) compared to the reference flour ET2 (4.50 mg/100 g). Among these, EF05, EF06, EF07, EF08, EE09, EE11, EE14 and EC15 flours had higher contents (10.50 - 15.95 mg/100 g) than that of the reference flour ET1 (10.01 mg/100 g). Palmyra news shoots flour didn't contain the vitamin E (Table 2).

3.2 Grouping of Samples According to Vitamins

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).

3.2.1 Principal component analysis (PCA)

Fig. 1.A shows the circle of correlations of the factorial axes F1 and F2, which express 89.96% of the total variability of the studied parameters. The component F1 with an eigenvalue of 4.04, expresses 57.67% of the variance. It is predominantly established by positive correlations with the vitamin C, vitamin B1, vitamin B2 and vitamin B6 contents and a negative correlation with the β-carotene content. The component F2, with its own value 2.26, expresses 32.29% of the variance is mainly formed by vitamin B9 and vitamin E with negative correlations (Table 4).

The characteristics and samples projections in the formed plan by the components F1 and F2 highlight three classes of flour. Class 1 consists essentially of commercial flours ET1 and ET2. These are distinguished by higher contents of vitamin C, vitamin B1, vitamin B2 and vitamin B6 than the derived values from produced composite flours and Palmyra young shoots flour. Class 2 contains composite flours samples. They provide higher contents of β-carotene, vitamin B9 and vitamin E than those of control flours and Palmyra new shoots flour (Fig. 1). *Borassus aethiopum* represents the third class which didn't contain vitamins B6 and E. It contains the lowest contents in other revealed vitamins except the vitamin C (that is statistically identical to those of the flours composite), and vitamin B1.

3.2.2 Hierarchical ascending classification

Hierarchical classification also reveals three classes of the flours samples, with the Euclidean distance from aggregation of 335. Class 1 represents commercial flours ET1 and ET2. Samples in this class are distinguished by higher levels of vitamin C, vitamin B1, vitamin B2 and vitamin B6 than other analyzed samples. Class 2 contains samples of composite flours. Class 2 samples have the highest β-carotene, vitamin B9 and vitamin E values. Class 3 didn't contain vitamins B6 and E and had the lowest contents in other revealed vitamins (Fig. 2).

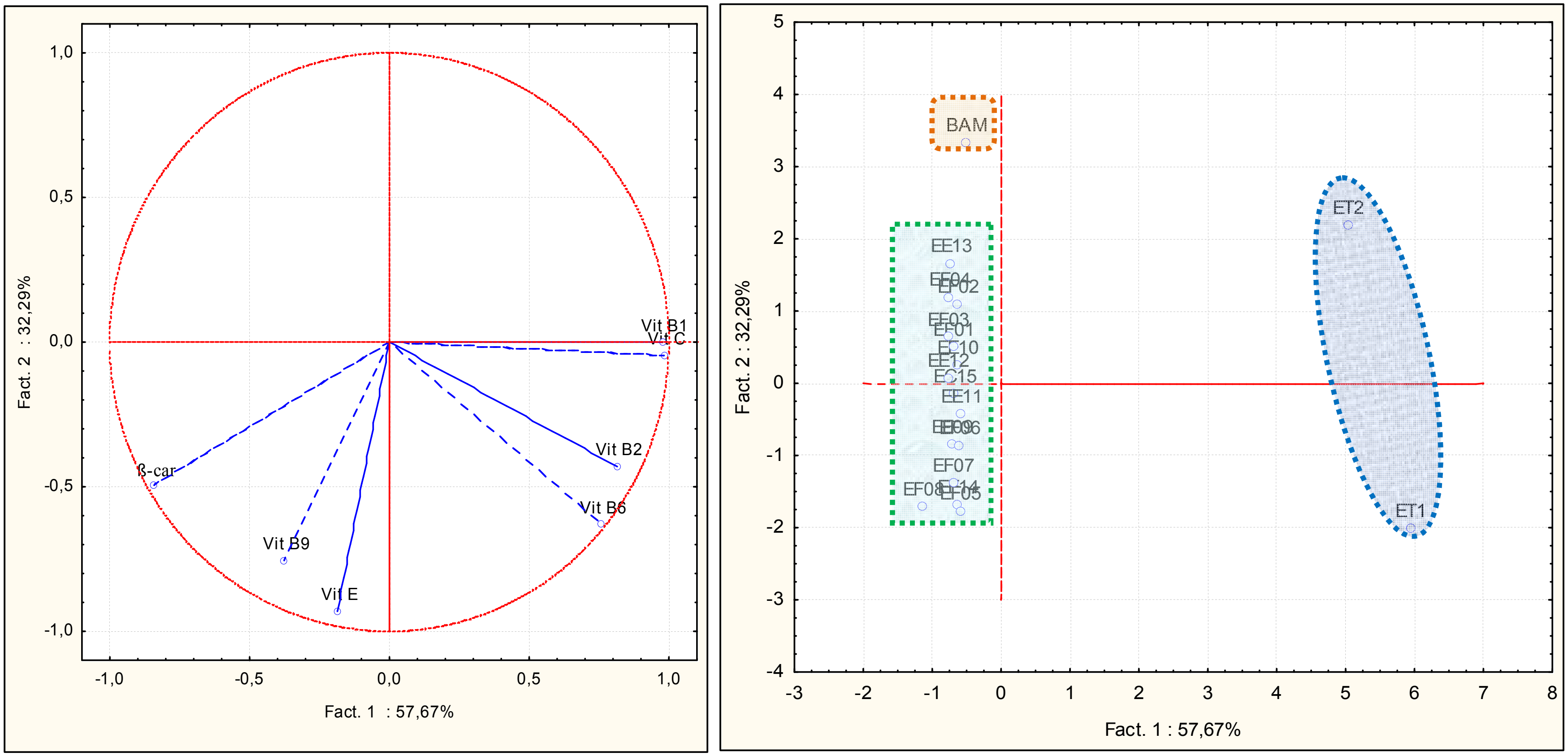
3.3 Estimated Intakes of Vitamins in 1- to 2-year-old

Sensory evaluation tests of the slurries prepared from the composite flours studied made it possible to retain flours EF07 and EE09 having exhibited the most interesting sensory characteristics. The quantities of vitamins provided by composite flours (EF07 and EE09), reference flours (ET1 and ET2) and *B. aethiopum* flour are evaluated.

Table 3. Contents in vitamins of the studied flours

Flour	Water-soluble vitamins					Fat-soluble vitamins	
	Vitamin C (mg/100 g DM)	**Vitamin B1 (mg/100 g DM)**	**Vitamin B2 (mg/100 g DM)**	**Vitamin B6 (mg/100 g DM)**	**Vitamin B9 (mg/100 g DM)**	**B-carotene (ER/100 g DM)**	**Vitamin E (mg/100 g DM)**
BAM	26.71±0.72^{c}	0.34±0.01^{c}	0.13±0^{k}	0.00^{l}	0.16±0.00^{g}	648.33±1.24^{i}	0.00^{p}
EF01	24.70±1.24^{c}	0.24±0.02^{d}	0.41±0.02^{h}	0.20±0.01^{hi}	0.24±0.01^{cdef}	793.33±0.30^{efg}	8.40±0.05^{k}
EF02	25.13±0.82^{c}	0.27±0.01^{d}	0.35±0.01^{i}	0.16±0.01^{ij}	0.22±0.01^{f}	765±0.17^{fgh}	6.73±0.04^{m}
EF03	24.15±1.30^{c}	0.22±0.01^{d}	0.40±0.01^{h}	0.18±0.01^{ij}	0.25±0.02^{bcdef}	755±0.07^{fgh}	7.56±0.07^{l}
EF04	24.67±1.18^{c}	0.24±0.02^{d}	0.34±0.02^{i}	0.14±0.00^{jk}	0.24±0.01^{def}	730±0.18^{gh}	5.88±0.05^{n}
EF05	24.18±1.99^{c}	0.23±0.01^{d}	0.65±0.03^{c}	0.38±0.02^{bc}	0.27±0.03^{bcd}	960±0.44^{a}	15.95±0.08^{a}
EF06	24.38±0.62^{c}	0.25±0.01^{d}	0.55±0.04^{e}	0.32±0.03^{de}	0.25±0.01^{bcdef}	905±0.32^{abc}	13.10±0.09^{d}
EF07	23.58±1.28^{c}	0.21±0.01^{d}	0.60±0.01^{d}	0.35±0.02^{cd}	0.28±0.01^{bc}	903.33±0.18^{abc}	14.27±0.07^{c}
EF08	24.13±1.95^{c}	0.22±0.01^{d}	0.52±0.02^{e}	0.28±0.01^{ef}	0.41±0.03^{a}	858.33±0.11^{cde}	11.76±0.08^{f}
EE09	23.88±0.83^{c}	0.21±0.01^{d}	0.55±0.01^{e}	0.30±0.02^{ef}	0.27±0.01^{bcd}	871.67±0.07^{bcde}	12.59±0.14^{e}
EE10	24.67±1.52^{c}	0.25±0.02^{d}	0.44±0.02^{gh}	0.23±0.01^{gh}	0.24±0.01^{cdef}	813.33±0.24^{def}	9.24±0.06^{j}
EE11	24.77±1.68^{c}	0.26±0.01^{d}	0.51±0.02^{ef}	0.28±0.01^{ef}	0.24±0.01^{cdef}	880±0.17^{bcd}	11.76±0.10^{f}
EE12	24.02±1.06^{c}	0.21±0.02^{d}	0.45±0.01^{g}	0.23±0.01^{gh}	0.26±0.01^{bcde}	798.33±0.15^{efg}	9.50±0.07^{i}
EE13	24.90±0.88^{c}	0.25±0.01^{d}	0.29±0.01^{j}	0.11±0.01^{k}	0.23±0.02^{ef}	703.33±0.09^{h}	4.54±0.03^{o}
EE14	23.88±1.49^{c}	0.22±0.01^{d}	0.64±0.03^{c}	0.38±0.02^{bc}	0.27±0.01^{bcd}	946.67±0.11^{ab}	15.62±0.08^{b}
EC15	24.35±1.17^{c}	0.23±0.01^{d}	0.48±0.01^{fg}	0.26±0.01^{fg}	0.25±0.01^{bcdef}	833.33±0.14^{cdef}	10.50±0.04^{g}
ET1	60.03±3.17^{a}	1.00±0.05^{a}	1.00±0.04^{b}	1.00±0.05^{a}	0.29±0.01^{b}	445±0.07^{j}	10.01±0.08^{h}
ET2	46.20±1.63^{b}	0.70±0.06^{b}	1.09±0.01^{a}	0.40±0.03^{b}	0.07±0.01^{h}	111.67±0.01^{k}	4.50±0.06^{o}
F	127.02	279.24	331.72	294.19	60.19	121.92	6733.26
P	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**

DM, dry matter; EF, factorial essay; EE, star essay; EC, essay at the centre; BAM, Borassus aethiopum Mart.; VUW, Vigna unguiculata Walp.; MOL, Moringa oleifera Lam.; ET1, sample control 1; ET2, sample control 2; Composite flours with respective percentages of BAM, VUW and 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; EE10, 72/17/11; EE11, 75/11/14; EE12, 61.4/27.3/11.3; EE13, 73/21.6/5.4; EE14, 62.8/18.6/18.6; EC15, 67.5/20/12.5; 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 vitaminic parameters (A) and samples (B) of the studied flours

Vit, vitamin; β-car, β-carotene; EF, factorial essay; EE, star essay; EC, essay at the centre; BAM, Borassus aethiopum Mart.; VUW, Vigna unguiculata Walp.; MOL, Moringa oleifera Lam.; Composite flours with respective percentages of BAM, VUW and 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; EE10, 72/17/11; EE11, 75/11/14; EE12, 61.4/27.3/11.3; EE13, 73/21.6/5.4; EE14, 62.8/18.6/18.6; EC15, 67.5/20/12.5

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

Components	F1	F2
Eigenvalue	4.04	2.26
Variability expressed (%)	57,67	32.29
Cumulative variability expressed (%)	57.67	89.96
Vitamin C	**0,98**	-0,05
β-carotene	**-0,84**	-0.49
Vitamin B1	**0,98**	-0.00
Vitamin B2	**0,81**	-0.43
Vitamin B6	**0,76**	-0.63
Vitamin B9	-0,38	**-0.75**
Vitamin E	-0,19	**-0.93**

Estimated vitamins intakes of flours EF07 and EE09 are significantly higher than those of the flour of *B. aethiopum*, except from vitamins C and B1. The flour EF07 contains daily vitamin B2 (1.5 mg/day), vitamin B6 (0.88 mg/day), vitamin B9 (0.7 mg/day), β-carotene (2258.33 ER/day) and vitamin E (35.68 mg/day) higher while Palmyra flour contains the lowest values (vitamin B2 (0.33 mg/day), vitamin B9 (0.4 mg/day) and β-carotene (1620.83 ER/day)).These two composite flours provide the same amount of vitamin B1 (0.53 mg/day). The flour EE09 provides vitamins B2, B6, B9, E and β-carotene with respective intakes of 1.38, 0.75, 0.68, 31.48 mg/day and 2179.18 ER/day.

Composite flours EF07 and EE09 provide also more β-carotene and vitamin E than the reference flours ET1 (1112.5 ER/day and 25.03 mg/day) and ET2 (279.18 ER/day and 11.25 mg/day), respectively. On the other hand, reference flours ET1 and ET2 have higher intakes of vitamin C (150.08 and 115.5 mg/day), vitamin B1 (2.5 and 1.75 mg/day), vitamin B2 (2.5 and 2.73 mg/day) and vitamin B6 (2.5 and 1 mg/day) (Table 5).

The daily estimated contributions in vitamins B2, B6, B9, E and β-carotene of the composite flours EF07 and EE09 are more high than those recommended by the mixed committee FAO/OMS and WHO.

Thus, the vitamins provided by composite flours EF07 and EE09 cover the daily intake of a 1- to 2-year-old recommended by mixed committee FAO/OMS and WHO, except vitamins C and B1 of which contributions are nevertheless very near of those of the recommendations.

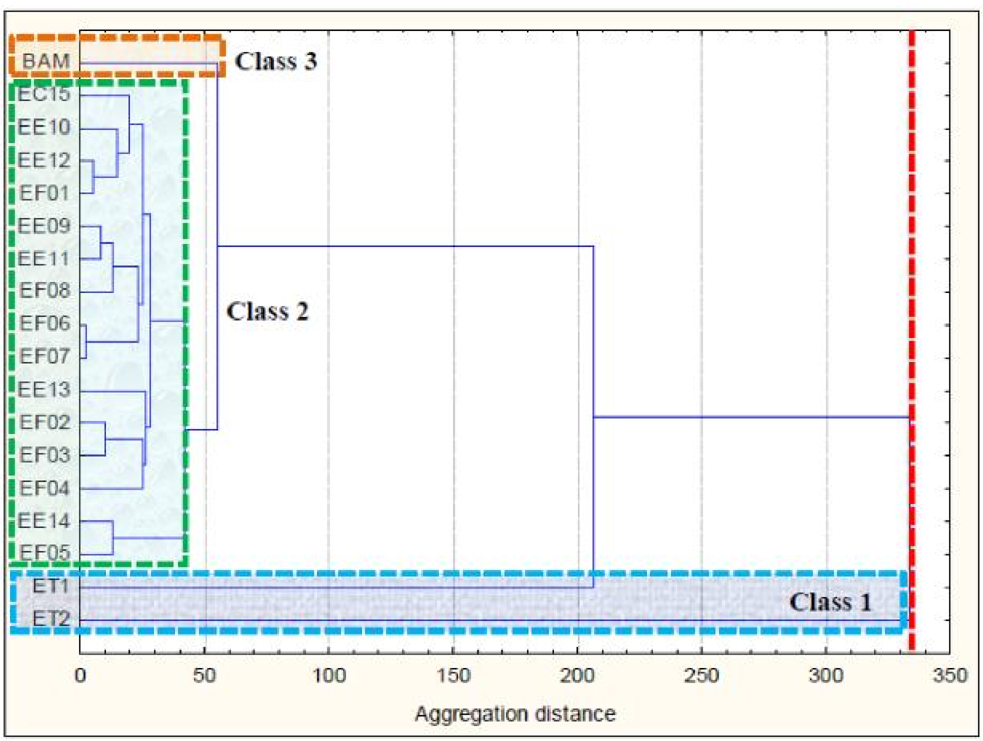


Fig. 2. Hierarchical classification of meals studied according to vitaminic characteristics

Table 5. Estimated daily intake in vitamins resulting from the consumption of 250 g of flour by a 1- to 2-year-old

Flour	Water-soluble vitamins										Fat-soluble vitamins			
	Vitamin C (mg/day)		Vitamin B_1 (mg/day)		Vitamin B_2 (mg/day)		Vitamin B_6 (mg/day)		Vitamin B_9 (mg/day)		β-Carotene (ER/day)		Vitamin E (mg/day)	
	DRI		**DRI**		**DRI**		**DRI**		**DRI**		**DRI**		**DRI**	
BAM		66.78		0.85		0.33		-		0.4		1620.83		-
EF07		58.95		0.53		1.5		0.88		0.7		2258.33		35.68
EE09	**60**	59.7	**0.6**	0.53	**0.8**	1.38	**0.7**	0.75	**0.16**	0.68	**400**	2179.18	**6**	31.48
ET1		150.08		2.5		2.5		2.5		0.73		1112.5		25.03
ET2		115.5		1.75		2.73		1		0.18		279.18		11.25

BAM: B. aethiopum Mart.; 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

4. DISCUSSION

The results indicate that enrichment significantly ($p < 0.001$) contributed to increase vitamin contents. This could be explained by the fact that *Moringa oleifera* used for fortification would have high micronutrient contents. Also, cowpea would contribute to the improvement of the vitamin content of *B. aethiopum* young shoots flour. These results are similar to those of other studies that present *Moringa oleifera* and cowpea as plant and natural reserves of micronutrients and proteins [23,24,16,15].

The results showed that, except vitamins C and B1, the composite flours obtained all had higher contents of β-carotene and vitamins B2, B6, B9 and E compared to the B. aethiopum new shoots flour produced in an artisanal way and consumed by certain populations. Moreover, this supplementation permitted to obtain flours with a high content of β-carotene and vitamin E than those of the control flours.

Beta-carotene enhances immune defences against infections [25]. Prolonged deficiency can cause paediatric blindness and severe infections that are often fatal in children [26]. As for vitamin E, it is an antioxidant involved in the protection of tissues and skin against oxidation and infections. The study of Okwu [27] showed that it protects cells against carcinogenesis. These composite flours also contain high amounts of vitamin B9 compared to that of the control flour ET2. This vitamin is essential for the formation of red blood cells and necessary for the proper functioning of the central nervous system [28]. Vitamin B9 helps to correct maternal anaemia [29]. Some factors such as hormonal contraception, repeated pregnancies, excessive alcohol and tobacco consumption may exacerbate the consequences its deficiency [30]. These flours could be promoted in the diet of Prevention of various pathologies related to food, in particular those induced by a deficiency of vitamin A and those resulting from oxidative stress [31,32].

On the other hand, composite flours have low levels of water-soluble vitamins C, B1, B2 and B6 compared to those of the control flours. However, the vitamin C content of these flours is higher than that recommended (2.3 mg/100 kcal) [33] for infantile flours. Vitamin B1 is involved in the oxidative decarboxylation of ketoacids and transketolization [34]. The vitamin B1 lack can cause asthenia, anorexia, vomiting and especially beriberi [35]. Vitamin B2 is involved in energy metabolism, contributing to tissue growth and repair, hormone production, and red blood cell structure. Vitamin B6 is a coenzyme involved in the metabolism of proteins, amino acids, glycogen, and neurotransmitter synthesis. It also contributes to the formation of antibodies and red blood cells [36].

The average daily quantity of flour consumed by a 1- to 2-year-old in Africa is 250 g [37]. During the intake of the same quantity, the estimated daily intakes in 1- to 2-year-old were much higher in composite flours (EF07 and EE09) compared to flour of *B. aethiopum*. This distribution shows the importance of the enrichment of *B. aethiopum* young shoots flour to the powders of *M. oleifera* leaves and cowpea seeds. Indeed, the incorporation of the powders of *M. oleifera* leaves and cowpea beans into the flour of young shoots of *B. aethiopum* significantly contributed to increase the vitamins contents. Composite flours EF07 and EE09 have the vitamin characteristics comparable to industrial infantile flours. Besides, they cover the daily recommended intake in vitamins in a 1- to 2-

year-old by FAO/WHO [38] and WHO [39]. Composite flours EF07 and EE09 can meet the nutritional needs of populations, especially children. Healthy vitamin intakes could help meet the ever-increasing needs of a 1- to 2-year-old and be beneficial in combating malnutrition.

5. CONCLUSION

Flours studied in this study present very varied vitamin compositions. The composite flours have the highest β-carotene, vitamin B9 and vitamin E values while reference flours are distinguished by higher contents of vitamin C, vitamin B1, vitamin B2 and vitamin B6. However, Palmyra new shoots flour didn't contain vitamins B6 and E. Moreover, it contains the lowest contents in vitamins B2 and B6. The enrichment of *B. aethiopum* new shoots flour to the *Moringa oleifera* leaflets and Cowpea beans powders is a considerable improvement of the nutritive characteristics of flour. Among these composite flours, the EF07 and EE09 flours have the vitamin characteristics comparable to reference flours, and cover the vitamin requirements of 1- to 2- year-olds. These 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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