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Assessement of Vitamins Contents of Almonds Deriving From *Terminalia catappa* L. (Combretaceae) Produced in Côte d'ivoire

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Authors' contributions

This work was carried out in collaboration between all authors. Author DTE designed the study, wrote the protocol, fitted the data and wrote the first draft of the manuscript. Author KNY performed the statistical analysis, checked the first draft of the manuscript for submission and revised the manuscript. Authors CA and SD managed the literature and assisted the experiments implementation. Author BGHM expertized the results interpretations. All authors read and approved the submitted manuscript.

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ABSTRACT

Aims: To assess the vitamins nutrients in almonds deriving from fruits of *Terminalia catappa* L. (Combretaceae) cultivated in Côte d'Ivoire.
Study Design: Mature fruits of *Terminalia catappa* harvested from various regions of Côte d'Ivoire, and then dried for allowing extraction of their almonds. Vitamins investigated from the almonds

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powder and their daily intake assessed according to the mean daily consumption of *Terminalia catappa*.
Place and Duration of Study: Laboratory of Biochemistry and Food Sciences, Biochemistry Department of Biosciences Unit, Félix Houphouet-Boigny University, between October and December 2015.
Methodology: Total of 540 kg *Terminalia catappa* dry fruits were gathered from suppliers in collecting regions. The dry fruits were opened using nutcracker for their almonds extraction. The extracted almonds were oven-dried, crushed, put into polyethylene bags, and then kept into a desiccator till analyses.
Results: The almonds displayed many essential vitamins, namely ß-carotene, tocopherol (vit E), niacin (vit B3), pyridoxine (vit B6), thiamine (vit B1), and folic acid (vit B9). The β-carotene and vit E (liposoluble vitamins) recorded respective contents of 1.25 ER/100 g and 1.19 mg/100 g. Regarding vit B3, vit B6, vit B1, and vit B9 (hydrosoluble vitamins) the studied almonds provided 116.4 µg/100 g; 38.31 µg/100 g, 2.75 µg/100 g, and 0.16 µg/100 g, respectively. The estimated daily intakes of the vitamins assessed are 0.012 ER/day (ß-carotene), 0.002 µg/day (vit B9), 0.012 µg/day (vit E), 0.03 µg/day (vit B1), 0.38 µg/day (vit B6), and 1.16 µg/day (vit B3).
Conclusion: The study showed the richness of vitamins essential in the almonds of *T. catappa.* The consumption of such vegetables could rely in nutritional profit for the human health and can therefore be recommended in the human diet.

Keywords: Almonds; vitamin contents; daily intake; Terminalia catappa; Côte d'Ivoire.

1. INTRODUCTION

Terminalia catappa belongs to the Combretaceae plant family originated from the Southern Asia. Producing fruits or almond nuts, this vegetable species was introduced in Côte d'Ivoire during the colonization era, through urban ornamentation [1]. *Terminalia catappa* usually reaches 8 m height at the adult stage and its green fruits turn to yellow at the full maturity. This plant has been the subject of many research attempts focusing the therapeutic and phytochemical properties of leaves. Thus, Masuda et al. [2] reported that the leaves' extracts of *Terminalia catappa* record anti-carcinogenic and antioxidant activities. Lin et al. [3] and Wang et al. [4] also stated on the antioxidant effect of those leaves which are often used as analgesic raw material. Moreover, Teotia and Singh [5] and Nagappa et al. [6] highlighted their inhibitory action against the raising in the blood glucose levels.

The fresh almonds of *Terminalia catappa* record a firm flavor: they are deeply delicious and appreciated and consumed by populations, especially children. According to Biego et al. [7], these almonds can be eaten as aperitif in various salty forms. In fact, the *Terminalia catappa* fruits are with greater nutritional interests. They are richer in essential minerals as potassium, magnesium, calcium, phosphorous, sodium, and iron with significant contents [8,9]. Dau et al. [10] also revealed many vitamin compounds in the *Terminalia catappa* fruits, namely ß-carotene (21.66 ER/100 g) and vitamins C (0.05 mg/100 g) and E (19.66 mg/100 g). The nutritional properties of such bio-molecules are widely reported. Indeed, vitamins have important function for the body and their intake via foodstuffs is highly recommended since they don't record significant biosynthesis in the organisms from the animal kingdom. According to Okwu [11], vitamins protect cells from carcinogenesis. The vitamins deficiency from the food diet sometimes results in nutritional disorders as beri beri, kwashiorkor, rachitism, mucous and cutaneous lesions, anemia, etc. For fitting the vitamins needs of the body, the consumption of foodstuffs richer in vitamins and found in the surrounding immediate environment of populations would be promoted.

The *Terminalia catappa* fruits are known to be consumed by some populations thanks to their sweet taste. Hence, the consumption of almonds as vegetables is growing in the human diet. Yet, fewer studies focus on the *Terminalia catappa* almonds and their vitamins are not yet soundly investigated, especially in Côte d'Ivoire where this vegetable is more produced in the western region. This work was carried out to assess the main vitamins in *Terminalia catappa* almonds and their daily intakes in order to promoting this product as functional foodstuff.

2. MATERIALS AND METHODS

2.1 Plant Material

The vegetable material consisted of dried ripe fruits from *Terminalia catappa* collected from different regions of Côte d'Ivoire.

2.2 Sampling

The mature dry fruits of *Terminalia catappa* were collected from October to December from producers in the Ivorian regions of Tonkpi (Man, Danane) and Guemon (Duékoué). Per city, three (3) suppliers were considered, providing 60 kg of dried fruits each. Thus, a total of 540 kg of dried fruits of *Terminalia catappa* were collected, conveyed at the laboratory and used for nutritional analyses.

2.3 Processing of the Dry Fruits of *Terminalia catappa*

The flow chart in the *Terminalia catappa* fruits processing is displayed in Fig. 1. The dried fruits were opened for almonds removal using nutcracker. The extracted almonds were dried at 50°C for 48 h in an oven (MEMMERT, Germany). After ambient cooling, they were crushed using a Magimix crusher, before being stored in sealed polyethylene bags and kept into desiccators till analysis.

2.4 Determination of Vitamins

The concentrations of hydrosoluble vitamins (group B vitamins) and lipo-soluble vitamins were determined using a High Performance Liquid Chromatographic apparatus (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 vitamin used as standard.

2.4.1 Validation of vitamin analysis

The validation of vitamins measuring by HPLC was implemented using standard method providing by NFV03-110 [12]. This procedure consists of studies of the linearity from the standardization range, the determination of the limits of detection and quantification (LOD and LOQ), the relative standard deviation regarding the repeatability and reproducibility essays, and the yield of extraction resulting from the recovery of the added components.

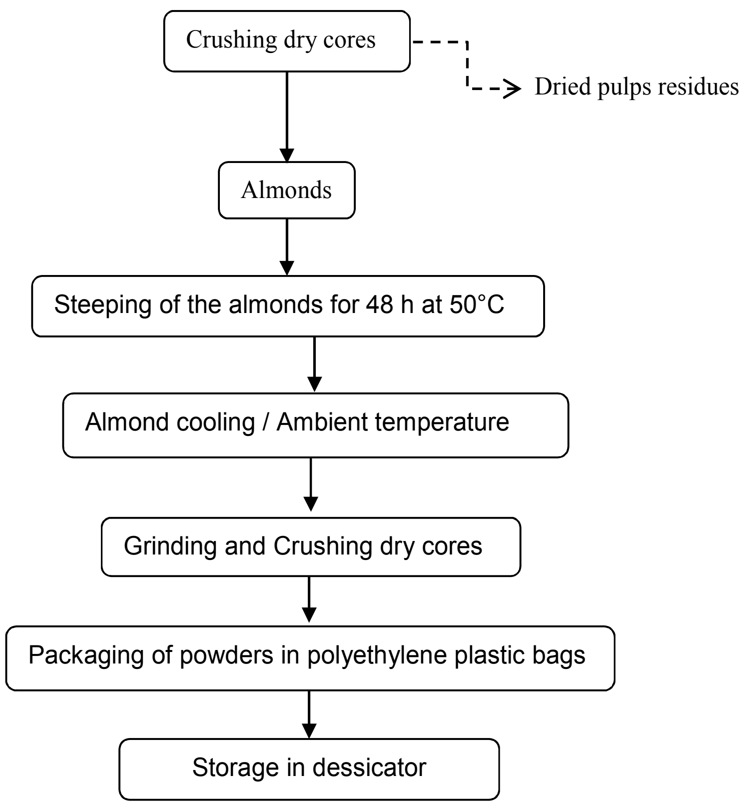

Fig. 1. Processing chart of dried fruits *Terminalia catappa* for resulting in almonds powder

2.4.1.1 Test of linearity

The linearity was tested between 0 and 125 µg/ml using 5 points of calibration: 0, 25, 50, 75, and 125 µg/ml. Five (5) distinct essays were performed.

2.4.1.2 Limits of detection and quantification

The limits of detection (LOD) and quantification (LOQ) were calculated with the standard vitamins considered. Ten (10) distinct essays were performed and the values were obtained considering the following formulas:

LOD = Average + 3 standard deviation
LOQ = Average + 10 standard deviation

2.4.1.3 Test of repeatability and reproducibility

For the repeatability, 10 analysis essays per standard vitamin were achieved, while the reproducibility assessment was worked with 5 separate tests per standard vitamin, using the HPLC system and 1 mg/ml each vitamin.

2.4.1.4 Determination of the vitamins recovering rate

Ten separate trials from reference vitamins samples were analyzed to assess the recovery percentage per vitamin using the HPLC system and standard concentrations of 5 mg/ml vitamin.

2.4.2 Preparation of almond samples for HPLC separation

Two (2) grams of almond powder sample were vigorously dissolved into five (5) mL of n-hexane solvent. The mixture was then centrifuged for 5 min at 3000 rpm. The organic phase was collected and the residue was similarly treated with for resulting in deeper extraction. The overall organic phases were gathered in total extract volume from which and an aliquot was injected into the HPLC system for vitamins separation.

The liposoluble vitamins were separated on a column Kromasil C18 of 30 * 4 mm (CIL CLUZEAV) in stainless steel. The mobile phase consisted of a HPLC grade acetonitrile/methanol mixture (80/20, v/v) provided by MERCK company (Germany). The column temperature was kept at 30°C, the elution duration was 35 min, and the flow rate was 1.2 ml/min.

The hydrosoluble vitamins were separated on a Zorbax column with silica support post-grafted in C18 (150 mm * 4.6 mm) with particles of 3 mm. The mobile phase was a mixture of ammonium acetate and methanol (grade HPLC, MERCK, Germany). The elution was programmed at 2 ml/min for 20 min.

Standard β-carotene and vitamin E were purchased from Fluka Chemie (Switzerland), while hydrosoluble vitamins were purchased from Sigma-Aldrich (UK).

Table 1 shows the concentrations of the standard vitamins used in the HPLC system.

2.5 Estimation of Vitamins Intake Deriving from the Almonds Consumption

Vitamins intake providing by the *Terminalia catappa* almonds was valued according to the *Codex Alimentarius* method accounting the vitamins concentrations recovered from foodstuffs and the daily food consumption from the 70 kg adult individual. The daily food consumptions are provided from studies achieved by the World Health Organization and Food and Agriculture Organization [13]. The contribution of the studied almonds in the recovering of the daily recommended intake of each nutrient was also estimated using standard formula [14].

Table 1. Concentration in standard vitamins for injection and optimal wavelengths

Standard vitamins	Concentration (µg/ml)	Wavelengts (nm)
vit B1	0.1 to 3.5	270
vit B3	0.1 to 7	265
vit B6	0.5 to 12	257
vit B9	0.5 to 5	280
ß-carotene	0.2 to 4.5	445
vit E	0.2 to 5.5	295

Estimated Daily Intake (EDI) = C × Q
Contribution (%) = (EDI × 100) / DRI

With: C, vitamin concentration measured; Q, food daily consumption; DRI, daily recommended intake

2.6 Statistical Analysis

The data were recorded using Excel software 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) regarding the type of vitamin. Means were compared using Student Newman Keuls post-hoc test at 5% significance level.

3. RESULTS

3.1 Validation Data

The results of the validation are presented in Table 2.

The coefficients of determination (R^2) obtained for the study of linearity are between 0.996 and 0.999 for the various standard vitamins.

The LOD values are between 25 µg/l and 135 µg/l, whereas the LOQ values vary from 83 µg/l to 449 µg/l. The relative standard deviations (coefficients of variation) resulting from the repeatability essays oscillate between 1.0±0.05% and 1.74±0.04% and those of the reproducibility tests fluctuate between 2.5±0.47% and 4.4±0.60%.

Regarding the extraction yield (recovering rate) of the standard minerals the rates oscillate between 96.8±0.14% and 100.5±0.07%.

3.2 Vitamin Contents In *Terminalia catappa* Almonds

Analysis of the vitamin concentration in *Terminalia catappa* almonds powder reveals the presence of the liposoluble vitamins (ß-carotene and vit E) and hydrosoluble vitamins (vit B1, vit B3, vit B6, and vit B9) as shown in Table 3.

The liposoluble vitamins are found in concentrations of 1.25±0.06 ER/100 g (ß-carotene) and 1.19±0.12 mg/100 g (vit E).

Regarding the hydrosoluble vitamins, the niacin (vit B3) is the most abundant nutrient accounting 116.4±5.77 µg/100 g, followed by the pyridoxine (vit B6) with a content of 38.3±1.20 µg/100 g. As for thiamine (vit B1) and folic acid (vit B9), the *Terminalia catappa* almonds display lower levels of 2.75±0.35 µg/100 g and 0.167±0.08 µg/100 g, respectively (Table 3).

3.3 Estimated Vitamins Intakes Deriving from the *Terminalia catappa* Almonds Consumption

The main intakes of the vitamins providing from the almonds assessed are shown in Table 4. The estimated daily intakes of hydrosoluble vitamins (vit B9, vit B1, vit B6, and vit B3) range from 0.002 µg/day to 1.16 µg/day. The consumption of *Terminalia catappa* almonds recovers 1% (vit B9) to 27.14% (vit B6) of the hydrosoluble vitamins recommendations.

The intakes of liposoluble vitamins (ß-carotene and vit E) are estimated at 0.012 ER/day and 0.012 µg/day, respectively. The current consumption of *Terminalia catappa* almonds results in contributions of 0.0015% and 0.1% of the respective ß-carotene and vit E food intake recommendations (Table 4).

Table 2. Main data of vitamins analytic validation by HPLC

Vitamins	Standard lines		Repeatability	Reproducibility	Extraction yield (%)	LOD (ng/ml)	LOQ (ng/ml)
	Equation	R^2	RSD (%)				
ß-carotene	Y=326,6x+152,9	0,99	1.5±0.12	4.4±0.60	98.7±0.88	125±0.69	416±0.25
vit E	Y=836,2x-5800	0.99	1.7±0.04	3.1±0.51	99.5±0.07	98±0.23	326±0.41
vit B1	Y=723.4x+1346	0.99	1.3±0.10	3.2±0.98	97.3±0.55	62±0.17	206±1.09
vit B3	Y=462.5x-331,5	0.99	1.4 ± 0.73	3.4 ± 0.63	99.1 ±0.18	64 ± 0.01	213 ± 1.62
vit B6	Y=550.9x+627.1	0.99	1.0 ±0.05	2.8 ± 0.41	97.7 ±0.59	25 ± 0.38	83 ± 0.47
vit B9	Y=942.4x-1615	0.99	1.2 ± 0.21	2.5 ± 0.47	98.6 ±0.44	33 ± 0.75	109 ± 0.15

R^2, coefficient of determination of standard lines, RSD, relative standard deviation, LOD/LOD, limit of determination/quantification

Table 3. Vitamins contents in almonds of *Terminalia Catappa* fruits from Côte d'Ivoire

Vitamins		Statistical parameter			
		Mean	RSD	F	P
Liposoluble	ß-carotene (ER /100 g)	1.25±0.06[c]	4.46	1109.6	< 0.001
	vit E (µg/100 g)	1.19±0.12[c]	9.91		
Hydrosoluble	vit B1 (µg/100 g)	2.75±0.35[c]	12.68		
	vit B3 (µg/100 g)	116.40±5.77[a]	4.96		
	vit B6 (µg/100 g)	38.31±1.20[b]	3.13		
	vit B9 (µg/100 g)	0.16±0.08[c]	46.99		

Means ± standard deviations with the same lowercase letters are statistically identical at 5% significance. F, value of the statistical Ficher test; P, probability value of the statistical test; RSD, relative standard deviation

Table 4. Vitamins recommendations and intake and contribution of the daily consumption (1 g) of *Terminalia catappa* almonds in their recovery

Vitamins	RDI (mg/day)	Estimated contribution	Contribution (%)
ß-carotene	800*	0.012 ER/day	0.0015
vit E	12	0.012 µg/day	0.1
vit B1	1.1	0.03 µg/day	2.73
vit B3	16	1.16 µg/day	7.25
vit B6	1.4	0.38 µg/day	27.14
vit B9	0.2	0.002 µg/day	1

RDI, recommended daily intake

4. DISCUSSION

The determination coefficients (R^2) got from the calibrations tests were close to 1, forecasting a quasi-linear estimation of the vitamin nutrients relating to their concentration in the product studied. The lower relative standard deviations (<5%) resulting from reproducibility and repeatability essays translate quite stability of the chromatography technique used (HPLC), which is as fitted as the full amount of each vitamin nutrient is revealed, as shown by the weak extraction defaults below 2.7% from the added vitamins. Thus, these characteristics highlight the reliability and precision of the outcomes in the vitamins contents determination using the HPLC device.

The studied *Terminalia catappa* almonds record 1.25±0.06 ER/100 g ß-carotene. But, higher contents of 151.6 ER/100 g and 21.66 ER/100 g have been reported for this vitamin by Dau et al. [10] and Udotong et al. [15], respectively, regarding the same vegetable. In the human's body, the ß-carotene acts in the eye retina allowing good vision, the growth and solidity of the cells tissues [16], the reproduction, and the immune defense against infections [16,17,18].

Hundred g of *Terminalia catappa* almonds analyzed are provided with 1.19 µg of the vitamin E, lower than the 19.66 µg/100 g reported thereabout by Dau et al. [10]. Nevertheless, the current study shows that these almonds are richer in vitamin E compared to the cashew nuts (0.289 µg/100 g) and Bambara peanuts (0.38 µg/100 g) which are other nuts products cultivated in Côte d'Ivoire [19,20]. Vitamin E protects the body against the effects of endogenous free radicals and exogenous free radicals originating from pollutants [21]. Besides, this vitamin is an antioxidant involved in the protection of tissues and skin from oxidation and infections, and supports the cells against the carcinogenesis [11].

From hydrosoluble vitamins, a mean vitamin B3 content of 116.4 µg/100 g is also found in the *Terminalia catappa* almonds investigated. Comparable vitamin B3 levels have previously been indicated from peanuts (98 µg/100 g) and cashew nuts (100 µg/100 g) by authors [22,23]. Vitamin B3 has significant role in the increase of HDL-cholesterol ("good cholesterol") and the decrease of LDL-cholesterol ("bad cholesterol") and the triglyceride levels [24,25].

The *Terminalia catappa* almonds also contain 38.3 µg of vitamin B6 in 100 g samples. From USDA reports [26], vitamin B6 is not found in Brazil nuts whereas it's valued at 100 µg/100 g cashew nuts. The vitamin B6 is coenzyme molecule that participates in the amino acids and glycogen metabolisms. It also contributes to the red blood cells synthesis prior to carry more oxygen [24]. From thiamine (vit B1) and folic acid (vit B9), the *Terminalia catappa* almonds displayed lower respective contents of 2.75 µg/100 g and 0.16 µg/100 g, insuring significant divergence in nutrients amounts from foodstuffs.

Higher contents in vitamins B1 (438 µg/100 g) and B9 (1.45 mg/100 g to 7 mg/100 g) have been reported from other nuts products as peanuts and cashew nuts [22,26]. The vitamin B1 has coenzyme role as thiamine pyrophosphate (TPP) in the metabolism of branched-chain carbohydrates and amino acids and participates in the formation of α-ketones [27,28]. Concerning vitamin B9, the biological role is related to the cells reproduction, the red blood cells synthesis, and the cancer prevention. Rimm et al. [29] show that the consumption of vitamin B9 foods reduces the risk of cardiovascular diseases.

According to WHO [13], the daily consumption of *Terminalia catappa* almonds is 1 g, which is lower compared to the full diet of 1018.1 g of food daily consumed per adult individual. This lower almonds consumption is explained by the food habits generally supported by starches and cereals from African populations, especially from Ivorians. These foods are often useless for vitamins interests and should be associated them with other vitamins food resources such as the almonds investigated in this study.

The *Terminalia catappa* almonds are below 0.01% of the daily consumed foods. Yet, the intake in liposoluble vitamins, especially vit E resulting thereabout is 0.1% DRI. From liposoluble vitamins these almonds contribute above 1% of the main recommendations. The *Terminalia catappa* almonds are therefore richer in vitamins and increasing their consumption could fit the populations vitamins needs.

5. CONCLUSION

This study revealed that the *Terminalia catappa* almonds are good source of liposoluble vitamins (vit B3, vit B6, vit B1, and vit B9) compared to liposoluble vitamins (ß-carotene and vit E). Although only 1 g of almonds is daily consumed in Côte d'Ivoire, they have significant contribution in vitamins intake, especially water-soluble vitamins. The increase in the daily consumption of almonds could successfully cover the vitamins needs for populations and fight malnutrition concerns such as avitaminosis. Regarding their vitamins richness, the *Terminalia catappa* almonds could be recommended in the human diet.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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