

Original Research Article

ASSESSMENT OF VITAMINS CONTENTS OF ALMONDS DERIVING FROM *TERMINALIA CATAPPA* L. (COMBRETACEAE) PRODUCED IN CÔTE D'IVOIRE.

ABSTRACT

Aims: To assess the vitamins and 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 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 Houphouët-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 β -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/100g and 1.19 mg/100g. Regarding vit B3, vit B6, vit B1, and vit B9 (hydrosoluble vitamins) the studied almonds provided 116.4 μ g/100g; 38.31 μ g/100g, 2.75 μ g/100g, and 0.16 μ g/100g, respectively. The estimated daily intakes of the vitamins assessed are 0.012 ER/day (β -carotene), 0.002 μ g/day (vit B9), 0.012 μ g/day (vit E), 0.03 μ g/day (vit B1), 0.38 μ g/day (vit B6), and 1.16 μ g/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 β -carotene (21.66 ER/100g) and vitamins C (0.05 mg/100g) and E (19.66 mg/100g). 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. MATERIAL 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.

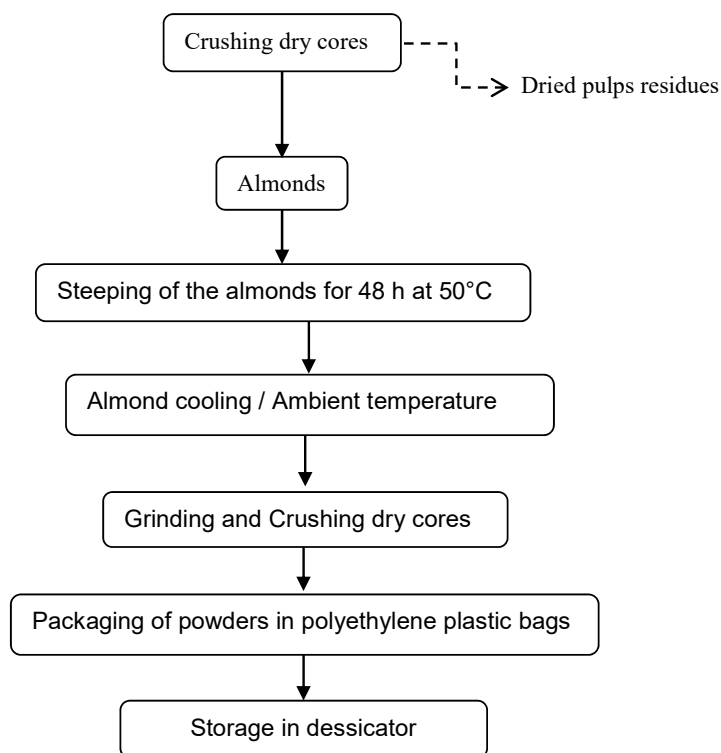


Figure 1: Processing chart of dried fruits *Terminalia catappa* for resulting in almonds powder

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.

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

96 The limits of detection (LOD) and quantification (LOQ) were calculated with the standard
97 vitamins considered. Ten (10) distinct essays were performed and the values were obtained
98 considering the following formulas:
99 $LOD = \text{Average} + 3 \text{ standard deviation}$
100 $LOQ = \text{Average} + 10 \text{ standard deviation}$

101

102 2.4.1.3 Test of repeatability and reproducibility

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

106

107 2.4.1.4 Determination of the vitamins recovering rate

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

111

112

113 2.4.2 Preparation of almond samples for HPLC separation

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

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

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

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

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

131

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

Standard vitamins	Concentration ($\mu\text{g/ml}$)	Wavelengths (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
β -carotene	0.2 to 4.5	445
vit E	0.2 to 5.5	295

133

134

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].

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

Contribution (%) = $(EDI \times 100) / DRI$

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 (β-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/100g (β-carotene) and 1.19±0.12 mg/100g (vit E).

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

3.3 Estimated vitamins intakes deriving from the *Terminalia catappa* almonds consumption

179 The main intakes of the vitamins providing from the almonds assessed are shown in Table 4.
 180 The estimated daily intakes of hydrosoluble vitamins (vit B9, vit B1, vit B6, and vit B3) range
 181 from 0.002 µg/day to 1.16 µg/day. The consumption of *Terminalia catappa* almonds recovers
 182 1% (vit B9) to 27.14% (vit B6) of the hydrosoluble vitamins recommendations.

183 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 (%)				
β-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

184 R², coefficient of determination of standard lines, RSD, relative standard deviation, LOD/LOQ, limit of
 185 determination/quantification
 186

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

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

188 Means ± standard deviations with the same lowercase letters are statistically identical at 5%
 189 significance. F, value of the statistical Fischer test; P, probability value of the statistical test; RSD,
 190 relative standard deviation
 191

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

Vitamins	RDI (mg/day)	Estimated contribution	Contribution (%)
β-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

194
 195

196 The intakes of liposoluble vitamins (β -carotene and vit E) are estimated at 0.012 ER/day and
197 0.012 $\mu\text{g/day}$, respectively. The current consumption of *Terminalia catappa* almonds results
198 in contributions of 0.0015% and 0.1% of the respective β -carotene and vit E food intake
199 recommendations (Table 4).

200

201 DISCUSSION

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

210 The studied *Terminalia catappa* almonds record 1.25 ± 0.06 ER/100g β -carotene. But, higher
211 contents of 151.6 ER/100g and 21.66 ER/100g have been reported for this vitamin by Dau *et al*
212 [10] and Udotong *et al* [15], respectively, regarding the same vegetable. In the human's
213 body, the β -carotene acts in the eye retina allowing good vision, the growth and solidity of
214 the cells tissues [16], the reproduction, and the immune defense against infections [16, 17,
215 and 18].

216 Hundred g of *Terminalia catappa* almonds analyzed are provided with 1.19 μg of the vitamin
217 E, lower than the 19.66 $\mu\text{g}/100\text{g}$ reported thereabout by Dau *et al* [10]. Nevertheless, the
218 current study shows that these almonds are richer in vitamin E compared to the cashew nuts
219 (0.289 $\mu\text{g}/100$ g) and Bambara peanuts (0.38 $\mu\text{g}/100\text{g}$) which are other nuts products
220 cultivated in Côte d'Ivoire [19, 20]. Vitamin E protects the body against the effects of
221 endogenous free radicals and exogenous free radicals originating from pollutants [21].
222 Besides, this vitamin is an antioxidant involved in the protection of tissues and skin from
223 oxidation and infections, and supports the cells against the carcinogenesis [11].

224 From hydrosoluble vitamins, a mean vitamin B3 content of 116.4 $\mu\text{g}/100\text{g}$ is also found in
225 the *Terminalia catappa* almonds investigated. Comparable vitamin B3 levels have previously
226 been indicated from peanuts (98 $\mu\text{g}/100\text{g}$) and cashew nuts (100 $\mu\text{g}/100\text{g}$) by authors [22,
227 23]. Vitamin B3 has significant role in the increase of HDL-cholesterol ("good cholesterol")
228 and the decrease of LDL-cholesterol ("bad cholesterol") and the triglyceride levels [24, 25].
229 The *Terminalia catappa* almonds also contain 38.3 μg of vitamin B6 in 100g samples. From
230 USDA reports [26], vitamin B6 is not found in Brazil nuts whereas it's valued at 100 $\mu\text{g}/100\text{g}$
231 cashew nuts. The vitamin B6 is coenzyme molecule that participates in the amino acids and
232 glycogen metabolisms. It also contributes to the red blood cells synthesis prior to carry more
233 oxygen [24]. From thiamine (vit B1) and folic acid (vit B9), the *Terminalia catappa* almonds
234 displayed lower respective contents of 2.75 $\mu\text{g}/100\text{g}$ and 0.16 $\mu\text{g}/100\text{g}$, insuring significant
235 divergence in nutrients amounts from foodstuffs. Higher contents in vitamins B1 (438
236 $\mu\text{g}/100\text{g}$) and B9 (1.45 mg/100g to 7 mg/100g) have been reported from other nuts products
237 as peanuts and cashew nuts [22, 26]. The vitamin B1 has coenzyme role as thiamine
238 pyrophosphate (TPP) in the metabolism of branched-chain carbohydrates and amino acids
239 and participates in the formation of α -ketones [27, 28]. Concerning vitamin B9, the biological
240 role is related to the cells reproduction, the red blood cells synthesis, and the cancer
241 prevention. Rimm *et al* [29] show that the consumption of vitamin B9 foods reduces the risk
242 of cardiovascular diseases.

243 According to WHO [13], the daily consumption of *Terminalia catappa* almonds is 1g, which is
244 lower compared to the full diet of 1018.1g of food daily consumed per adult individual. This
245 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.

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 (β -carotene and vit E). Although only 1g 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.

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