

Original Research Article

**THE ESSENTIAL VITAMINS CONTENTS OF
TERMINALIA CATAPPA L. (COMBRETACEAE)
ALMONDS CULTIVATED IN CÔTE D'IVOIRE.**

ABSTRACT

Aims: To assess the vitamins nutrients in almonds derived from fruits of *Terminalia catappa* L. (Combretaceae) cultivated in Côte d'Ivoire.

Study design: The almonds of *Terminalia catappa* extracted from the dry fruits harvested in different regions of Côte d'Ivoire. The concentration of the vitamins was determined and their contribution.

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: The dry fruits of *T. catappa* were opened using nutcracker. The extracted almonds were oven dried, crushed, put into polyethylene bags and then kept into a desiccator till analyses.

Results: The results revealed the presence of vitamins β -carotene, E, B3, B6, B1 and B9. The β -carotene and E vitamins are obtained with respective contents of 1.25 ER/100g and 1.19 mg /100g. Concerning the water-soluble vitamins B3, B6, B1 and B9, the recovered contents are respectively 116.4 μ g/100g; 38.31 μ g/100g, 2.75 μ g/100g and 0.16 μ g/100g. The estimated intakes of these vitamins evaluated fluctuate between 0.002 μ g/day (vitamin B9) and 1.16 μ g/day (Vitamin B3). For β -carotene, E, B1 and B6 vitamins they are 0.012 ER /day, 0.012, 0.03 and 0.38 μ g/day, respectively.

Conclusion: This study showed the richness of vitamins essential in the almonds of *Terminalia Catappa*. The consumption of almonds of *Terminalia catappa* would have a beneficial effect on human health and can be recommended in human diet.

Keywords: Almonds, vitamins, daily intake, *Terminalia catappa*, Côte d'Ivoire

1. INTRODUCTION

Terminalia Catappa belongs to the Combretaceae family, native to Southern Asia. Produced from fruits or almond nuts, it was introduced in Côte d'Ivoire thanks to colonization, through urban ornamentation [1]. *T. catappa* can reach 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 much research on the therapeutic and phytochemical properties of leaves. Masuda *et al* [2] reported that leaf extracts of *Terminalia catappa* possess anti-carcinogenic and antioxidant activities. Lin *et al* [3] and Wang *et al* [4] show that the leaves of *T. catappa* have antioxidant and anti-

inflammatory activities. Téotia and Singh [5] and Nagappa *et al* [6] also highlighted their inhibitory action on elevated blood glucose levels. The fresh almonds of this plant have a delicious taste and are consumed by children. Biego *et al* [7] revealed that the almonds of *T. catappa* can be eaten as an aperitif in more or less salty forms. Others studies showed richness in minerals as potassium, magnesium, calcium, phosphorous, sodium, and iron in appreciable proportions [8; 9], and also in vitamins β -carotene (21,66ER/100g), C (0, 05 $\pm$ 0, 00 mg/100g) and E (19,66 $\pm$ 0,2 mg/100g) [10]. Vitamins play an important role in the functioning of the body and are only bring by the food. According to Okwu [11], vitamins can also protect cells to carcinogenesis. Their absence in the diet sometimes causes nutritional diseases. To fill their deficit in the body, it is important to find other sources of foods rich in vitamins present in our immediate environment such *T. catappa*. Furthermore, work on the vitamin content of *T. catappa* almonds is not exhaustive.

In Côte d'Ivoire, fewer studies focus on the vitamin content of *T. catappa* almonds. However, consumption of almonds is growing in the human diet. It is in this context, this present work was initiated to assess the vitamins (water and fat-soluble), their contribution in almonds in order to propose them as a new source of vitamins for human diet.

2. MATERIAL AND METHODS

2-1 Plant material

The vegetable material consisted of dried ripe fruits from *T. catappa* collected from different regions of Côte d'Ivoire.

2-2 Sampling

The dried fruits of *T. catappa* were collected December to October in the regions of tonkpi (Man, Danane) and Guemon (Duékoué) of the Côte d'Ivoire from producers. In each city, 3 suppliers were considered. At these last, 60 kg of dried fruits were collected. Thus, a total of 540 kg of dried fruit of *T. catappa* were collected, escorted and crushed in the laboratory for analysis.

2-3 Treatment of dried fruits of *T. cappa*

The treatment diagram of the fruits of *T. catappa* is detailed in Figure 1. The dried fruits of *T. catappa* were opened using nutcracker. The extracted almonds were dried at 50° C. for 48 h in an oven (MEMMERT, Germany). After cooling, they were crushed (Magimix Crusher) before being stored in sealed polyethylene bags and then stored in a desiccators before analysis.

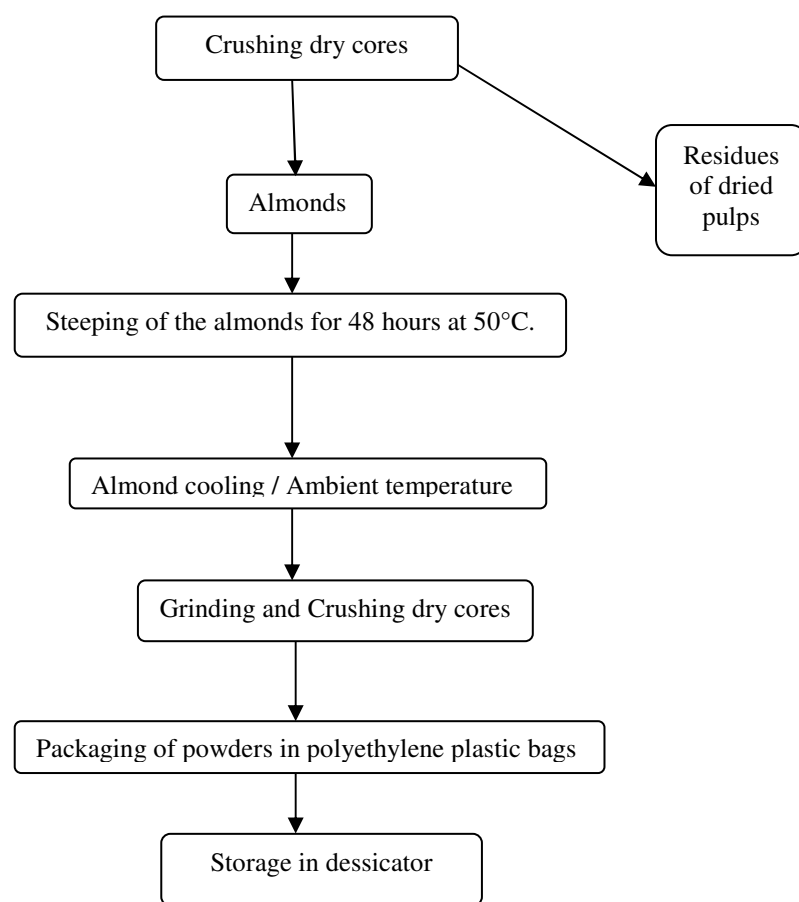


Figure 1: Treatment steps of dried fruits *T. catappa* to obtain almonds powder

2-4 Determination of vitamins

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.

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2.4.1 Preparation of samples for HPLC separation

99 Two grams of flour samples were extracted vigorously with five excess of n-hexane solvent
 100 followed by centrifugation in the cold for 5 min at 3000 rpm. The organic solvent was
 101 aspirated and saved. The residue was reextracted with the same solvent and the same
 102 steps were repeated until the extract was almost colorless. The total volume of the extract
 103 was recorded and an aliquot was injected in the HPLC system. Fat soluble vitamins were
 104 separated on a column Kromasil C18 of 30 X 4 mm (CIL CLUZEAV) in stainless steel. The
 105 mobile phase was a mixture acetonitrile / methanol (80/20, v / v), of HPLC grade and well
 106 furnished by MERCK (Germany). The column temperature was 30°C, the elution length was
 107 35 min and the flow rate was 1.2 ml/min.
 108 Water soluble vitamins were separated on a Zorbax column to silica support post grafted in
 109 C18 (150 mmX4.6mm) with particles of 3 mm. The mobile phase was a mixture of
 110 ammonium acetate and methanol, of grade HPLC and furnished by MERCK (Germany). The
 111 flow rate was programmed to 2 ml/min on a length of 20 min. Standard β -carotene and
 112 vitamin E were purchased from Fluka Chemie (Switzerland), while water soluble vitamins
 113 were purchased from Sigma-Aldrich (UK). Table 1 present the concentrations of the
 114 standard vitamins used for injection in the HPLC system.

115

116 **Table 1: Concentration for injection and wavelengths**

Vitamins	Concentration ($\mu\text{g/ml}$)	Wavelengths (nm)
B1	0.1 à 3.5	270
B3	0.1 à 7	265
B6	0.5 à 12	257
B9	0.5 à 5	280
β -carotene	0.2 à 4.5	445
E	0.2 à 5.5	295

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118 2.4.2 Validation of vitamin analysis

119 The validation of vitamins dosage by HPLC was been achieved by the application the
 120 NFV03-110 norm [12]. This procedure consists of studies of the linearity of the
 121 standardization range, the determination of the limits of detection and quantification, the
 122 calculation of the variation coefficient on tests of repeatability and reproducibility, as well as
 123 the calculation of the percentage of addition recovery measured.

124 2.4.2.1 Test of Linearity

125 The linearity was tested between 0 and 125 $\mu\text{g/ml}$ using 5 points of calibration: 0, 25, 50, 75
 126 and 125 $\mu\text{g/ml}$, five distinct tests were carried out.

127 2.4.2.2 Limits of detection and quantification

128 The limits of detection (LD) and quantification (LQ) were calculated with the standards of the
 129 various required vitamins. Ten (10) distinct tests were carried out and the values were
 130 obtained with the following formulas:

131 $\text{LD} = \text{Average (MX)} + 3 \text{ standard deviation}$

132 $\text{LQ} = \text{Average (MX)} + 10 \text{ standard deviation}$

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135 2.4.2.3 Test of repeatability and reproducibility

136 To test the repeatability, 10 trials of extract from a reference sample were analyzed by
137 HPLC. For reproducibility, 5 separate tests sample from a reference sample were analyzed
138 by HPLC at intervals of several days. The standard concentrations are 1 mg/ml.

139 2.4.2.4 Test of accuracy

140 Ten separate trials from reference samples were analyzed to assess the recovery rate by
141 the method used for determination of vitamins. The standard concentrations are 5 mg/ml.
142

143 2.5 Estimate of nutritive supply at the consumer

144 Vitamin supply have been estimated according to the method of the Codex Alimentarius that
145 takes into account the concentrations in vitamins recovered in the food and the daily
146 consumption of an adult individual of 70 kg of this food. These quantities of food are given by
147 World Health Organization studies [13]. The contribution of this food in daily requirement has
148 been calculated also from the values of daily recommended intakes [14].

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

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

151 **C:** Vitamin concentration measured

152 **Q:** food daily consumption

153 **DRI:** daily recommended intake.

154 2.6 Statistical analysis

155 The data were recorded with Excel file and statistically treated with Statistical Program for
156 Social Sciences (SPSS 22.0 for Windows).The statistical test consisted in a one-way
157 analysis of variance (ANOVA) with the type of vitamin. From each parameter, means were
158 compared using Student Newman Keuls post-hoc test at 5 % significance level.

159 3. RESULTS

160 3.1 Validation results

161 The results of the validation are presented in Table 2. The coefficients of determination
162 obtained for the study of linearity are between 0.996 and 0.999 for the various required
163 vitamins. The limits of detection lie between 25 µg/l and 135 µg/l, whereas the limits of
164 quantification vary from 83 µg/l with 449 µg/l. The coefficients of variation determined for the
165 repeatability oscillate between 1.0±0.05% and 1.74±0.04% and those of the tests of
166 reproducibility lie between 2.5±0.47% and 4.4±0.60%. As for the outputs of extraction of the
167 proportioned additions, the rates are consisted between 96.8±0.14% and 100.5±0.07%.

168 3.2 Vitamin concentrations recovered in almonds of *Terminalia catappa*

169 Analysis of the vitamin concentration of almonds of *Terminalia catappa* reveals the presence
170 of the fat-soluble vitamins (β-Carotene and E) and water-soluble (B1, B3, B6 and B9)
171 presented in Table 3. Fat-soluble vitamins, especially vitamin β-carotene and vitamin E are
172 found to have levels of 1.25±0,06ER/100g and 1.19±0,12mg/100g, respectively. Concerning
173 water-soluble vitamins, vitamin B3 is the most abundant with a content of 116.4± 5,77µg /
174 100g followed by vitamin B6 with a content of 38.3±1,20µg/100g. As for vitamins B1 and B9,
175 they show the lowest levels with a mean of 2.75±0,35µg/100g and 0.167±0,08µg/100g
176 respectively (Table 3).

177 **Table 2:** Tests results of vitamin analytic validation by HPLC

Vitamins	Standard lines		Repeatability	Reproducibility	Ext yield (%)	LOD (ng/ml)	LOQ (ng/ml)
	Equation	R ²	RSD (%)			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
E	Y=836,2x-5800	0.99	1.7±0.04	3.1±0.51	100.5±0.07	98±0.23	326±0.41
B ₁	Y=723.4x+1346	0.99	1.3±0.10	3.2±0.98	97.3±0.55	62±0.17	206±1.09
B ₃	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
B ₆	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
B ₉	Y=942.4x-1615	0.99	1.2 ± 0.21	2.5 ± 0.47	98.6 ± 0.44	33 ± 0.75	109 ± 0.15

178 *R², coefficient of determination of standard lines, RSD, relative standard deviation, LOD, limit of*
 179 *determination, LOQ, limit of quantification*
 180

181 **Table 3:** vitamins Concentration in almonds of *Terminalia catappa* fruit from Côte d'Ivoire.

Vitamins		Statistical parameter			
		Mean	RSD	F	P
Fat - soluble	β-carotene (ER /100g)	1,25±0,06 ^c	4,46	1109,6	< 0,001
	E (μg/100g)	1,19±0,12 ^c	9,91		
Water- soluble	B ₁ (μg/100g)	2,75±0,35 ^c	12,68		
	B ₃ (μg/100g)	116,40±5,77 ^a	4,96		
	B ₆ (μg/100g)	38,31±1,20 ^b	3,13		
	B ₉ (μg/100g)	0,16±0,08 ^c	46,99		

182 *Means ± standard deviations with the same lowercase letters are statistically identical at 5%*
 183 *significance. F, value of the statistical Ficher test, P, probability value of the statistical test; RSD,*
 184 *relative standard deviation.*
 185

186 3.3 Estimated Intakes among Adult Ivorian

187 The estimation of the intakes of fat-soluble and water-soluble vitamins is presented in Table
 188 4. The estimated intakes of water-soluble vitamins (B₉, B₁, B₆ and B₃) range from 0.002 μg
 189 / d to 1.16 μg / d. However, the intakes of fat-soluble vitamins (β-carotene and E) are
 190 estimated at 0.012 ER / d and 0.012 μg / d respectively.

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Table 4: Vitamins intake recommendations (mg / day) and contribution of *T. catappa* almonds (%) in their recovery

Vitamins	Estimated contribution	RDA	Contribution (%)
β-carotene	0,012 ER/d	800*	0,0015
E	0,012 µg/d	12	0,1
B1	0,03 µg/d	1,1	2,73
B3	1,16 µg/d	16	7,25
B6	0,38 µg/d	1,4	27,14
B9	0,002 µg/d	0,2	1

RDI: Recommended daily intake

DISCUSSION

The R^2 determination coefficients got from the calibrations tests were close to 1, forecasting a quasi-linear estimation of the vitamin 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 HPLC chromatography technique used, 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 chromatography technique.

The content of vitamin β-carotene in almonds of *Terminalia catappa* is 1.25 ± 0.06 ER/100g. With the same vegetable material Dau *et al* [10] and Udotong *et al* [15] reported high contents of 151.6 ER/100g and 21.66 ER/100g, respectively. Beta-carotene in the body acts two-levels, first in the retina of the eye and secondarily in the tissues to maintain the growth and solidity of the cells [16]. Also β-carotene intervenes in reproduction, growth, development of fetal vision, as well as in the preservation of ocular health and night vision of the mother. It strengthens the immune defenses against infections [17; 18]. For 100g of almonds of *Terminalia catappa* analyzed the vitamin E content is 1.19 µg /100g, lower than that of 19.66 µg/100g underlined by Dau *et al* [10]. Otherwise, this content (1.19 µg/100g) is greater than those (0.289 µg/100 g and 0.38 µg/100 g) reported by Ricard *et al* [19] and Yao *et al* [20] respectively in cashew nuts and Bambara peanuts from Côte d'Ivoire. This testifies to the richness of almonds in vitamin E compared to cashew nuts and bambara peanuts from Côte d'Ivoire. Vitamin E protects the body from the effects of endogenous free radicals and exposure to free radicals of exogenous origin from cigarette smoke and pollutants [21]. Also vitamin E is an antioxidant involved in protecting tissues and skin from oxidation and infections. It also protects cells against carcinogenesis [11].

Concerning vitamin B3, the almonds of *Terminalia catappa* contain 116.4µg/100g. Otherwise, Settaluri *et al* [22] and Dignan *et al* [23] reported levels of vitamin B3 for peanut and cashew nuts at 98 µg/100g and 100 µg /100g respectively. This showed the vitamin B3 richness of the almonds studied compared to peanuts and cashews. Vitamin B3 helps to significantly increase HDL ("good cholesterol"), lower LDL ("bad cholesterol") and reduce triglyceride levels [24; 25]. Analysis of 100 g almonds of *Terminalia catappa* revealed the presence of vitamin B6 with 38.3µg. According to U.S. Department of Agriculture [26], vitamin B6 is absent (0mg) in Brazil nuts and present in cashew nuts with 0.1mg /100g. Vitamin B6 called pyridoxine is a coenzyme that participates in the metabolism of amino acids and glycogen. Vitamin B6 also contributes to the formation of red blood cells and allows them to transport more oxygen [24]. The almonds of *Terminalia catappa* contain vitamin B1 with a content of 2.75µg/ 100g. This content (2.75 µg/100 g) is lower than that indicated (438 µg/100g) by Settaluri *et al* [22] in peanuts. Vitamin B1 plays the role of

coenzyme as thiamine pyrophosphate (TPP) in the metabolism of branched-chain carbohydrates and amino acids. Moreover, magnesium (Mg^{2+}) associates with thiamine precisely at the TPP to participate in the formation of α -ketols [27; 28]. Concerning vitamin B9, the almonds of *Terminalia catappa* contain 0.16 $\mu g/100$ g. Settaluri *et al* [22] and U.S. Department of Agriculture [26] reported levels of 1.450mg / 100g and 7mg / 100g of vitamin B9 respectively in peanut and cashew nuts. Vitamin B9 participates in cellular reproduction, in the synthesis of red blood cells and prevents the appearance of cancer. Rimm EB *et al* [29] show that consumption of vitamin B9-rich foods reduces the risk of cardiovascular disease compared to those with lower intakes. According to WHO [13], the daily consumption of *Terminalia catappa* almonds is 1g, which is very low compared to the 1018.1g of food consumed per day. This low consumption of almonds is explained by the eating habits generally based on starches and cereals in African populations in general and Ivorian in particular. These foods are usually low in vitamin so it would be important to associate them with other vitamin resources such as almonds. The intakes of water-soluble vitamins for consumption of 1g almonds fluctuate between 0.002 $\mu g/d$ and 1.16 $\mu g/d$. They have contributions all above 1% except vitamin B9 with 1%. This translated the richness of almonds in these vitamins. Concerning β -carotene and vitamin E, they have low overall intakes and contribute less than 0.5%. Thus an increase in the consumption of almonds could make it possible to cover the daily vitamin needs of the populations.

Conclusion

This study revealed that *Terminalia catappa* kernels are a good source of water-soluble vitamins (B3, B6, B1, and B9) compared to fat-soluble (β -carotene and E). Although consumption of almonds is very low (1 g/d) compared with the daily intake (1018.1 g/d), the intakes and contributions of water-soluble vitamins are important. The intakes and contributions of fat-soluble vitamins appear to be low, but compared to the amount of 1g of ingested almonds, they are considerable. Thus the increase in the daily consumption of almonds would better cover the needs of vitamins and especially to fight avitaminosis often cause of infant mortality. To the look of these vitamins richness's the almonds of *Terminalia catappa* could be recommended in the human food.

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