



Full paper/Mémoire

Chemical composition and nutritional properties of *Terminalia catappa* L. oil and kernels from Benin



Composition chimique et propriétés nutritionnelles des amandes et huile de fruits de Terminalia catappa L. du Bénin

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ABSTRACT

This work focuses on the physico-chemical characterization of kernels and oil of *Terminalia catappa* L. from Benin. The detailed physico-chemical properties are given including the nutritional composition and fatty acid profile, but also the phenolic content, phytochemical screening and antioxidant capacity which were determined for the first time. The kernel (100 g) contained 5.5 g of moisture, a high level of lipids (64.7–140.4 of Recommended Daily Intake (RDI)), proteins (36.0% RDI), sugars (6.0% RDI), and tannins (0.6%). The defatted kernels (100 g) contained high levels of manganese (184.8–236.1% RDI), magnesium (173.6–235.2% RDI), iron (89.7–201.9% RDI), zinc (87.9–120.9% RDI) and calcium (41.5% RDI), and contributed for 98.6% of RDI proteins. The kernel oil showed a high level of unsaturated fatty acids including oleic (27.1%) and linoleic acids (26.6%) and saturated fatty acids such as palmitic acid (40.0%) as well as several phytosterols and triterpenes. These kernels and their unsaturated oil are of interesting nutritional value but could also be used as a biofuel or lubricant. The presence of phenolic and terpenic derivatives may also explain at least in part their use in traditional medicine.

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R É S U M É

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La caractérisation physico-chimique de l'huile et des amandes de fruits de *Terminalia catappa* du Bénin a été réalisée. Les propriétés physico-chimiques ont été analysées, notamment la composition nutritionnelle et le profil en acides gras, mais aussi la teneur en polyphénols, le criblage phytochimique et l'activité antioxydante, qui ont été déterminées pour la première fois. Les analyses réalisées montrent que 100 g d'amandes contiennent 5,5 g d'humidité, un taux élevé de lipides (contribuant pour 63,7 à 140,4% de la dose journalière recommandée (DJR)), de protéines (36,0% de DJR), de sucres (6,0% DJR) et de tanins (0,6%). L'amande délipidée contient des taux élevés de manganèse (184,8–236,1% DJR), de magnésium (173,6–235,2% DJR), de fer (89,7–201,8% DJR), zinc (87,9–120,9% DJR) et calcium (41,5% DJR), et contribue pour 98,8% de la DJR en protéines. L'huile est riche en acides gras insaturés, tels que l'acide oléique (27,1%) et l'acide linoléique (26,6%), et en un acide gras saturé, l'acide palmitique (40,0%), et contient aussi des phytostérols et triterpènes. Ces amandes et cette huile de type insaturé ont une valeur nutritionnelle intéressante et peuvent aussi être utilisées comme biocarburants ou lubrifiants pour moteurs. La présence de composés phénoliques et terpéniques peut aussi expliquer, au moins en partie, leur usage en médecine traditionnelle.

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1. Introduction

Nuts have been the food of man from the earliest times in many parts of the world [1]. The interest on nuts is based on their nutritive value as they contain a significant amount of high quality proteins and vital minerals [2]. The superior quality of nut proteins makes them good substitutes for animal food, but nuts are also good sources of edible oils and fats [3].

Terminalia catappa (Tropical almond) is a large, spreading tree now distributed throughout the tropics in coastal environments. The tree is tolerant to the strong winds, the salt spray, and moderately to a high salinity in the root zone. It mainly grows in freely drained, well aerated, sandy soils. The species has traditionally been very important for coastal communities, providing a wide range of non-wood products and services. It has a spreading, fibrous root system and plays a vital role in coastline stabilization [4]. It is widely planted throughout the tropics, especially along sandy seashores, for shade, ornamental purposes, and edible nuts. The timber makes a useful and decorative general-purpose hardwood and is well suited for conversion into furniture and interior building timbers. Fruits are produced from about 3 years of age, and the nutritious, tasty seed kernels may be eaten. Tropical almond, easily propagated from seed, grows rapidly and blooms with minimal maintenance in appropriate environments. Selected cultivars of the species warrant wider commercial planting for joint production of timber and nuts. The tree has a demonstrated potential to naturalize in coastal plant communities, but not to adversely dominate such communities [4]. The productivity and marketing of cultivars with large and/or soft-shelled nuts need to be assessed. There is also a need for experimental work to develop vegetative propagation techniques and more efficient techniques for processing fully mature fruits including drying, storage, and cracking of nuts. Nutrient potential has been previously studied and shows the high protein content, fiber, fat, minerals and tannin content of the kernels from different origins [5–8]. The mineral profile of this kernel revealed many important

minerals as magnesium, calcium, phosphorous and iron in appreciable proportions [5–8]. It has aphrodisiac activity and may be useful in the treatment of certain forms of sexual inadequacies such as premature ejaculation [9]. It can also be used in the treatment of liver cancer and diabetes [10]. Extracts from the leaves and bark of the plant are also reported to have anti-HIV, anti-carcinogenic, and hepatoprotective properties [10]. Generally, oil is extracted from kernels by pressing or using a solvent [11,12] and contained more than 50% of unsaturated fatty acid and in high proportion, palmitic, oleic, linoleic and stearic acids [8,13,14]. It has also been shown that this oil has acceptable physico-chemical properties to be used as a biodiesel [15]. Information was available on the kernels from African countries such as Somalia [13], Congo [8], Ghana [10] and Nigeria [3,5]. But to our knowledge there are no reports to date concerning the *T. catappa* L. oil and kernels from Benin. The aim of this study is to describe the chemical composition and the nutritional properties of *T. catappa* L. oil and kernels from Benin.

2. Materials and methods

2.1. Chemicals and drugs

Dimethyl sulfoxide (DMSO), 1,1-diphenyl-2-picrylhydrazole (DPPH), 40 component fatty acid methyl esters (FAMES), and Folin-Ciocalteu's phenol reagent were purchased from Sigma-Aldrich (Steinheim, Germany), Acros Organics (New Jersey, USA), and Fluka Chemie (Buchs, Switzerland). Hexane was purchased from Fluka Chemie, anhydrous Na₂SO₄ from UCB (Brussels, Belgium) and absolute ethanol and absolute methanol from Labotec (Brussels, Belgium). All compounds and solvents were of analytical grade. Hide powder CRS was obtained from European Pharmacopoeia (EDQM, Strasbourg Cedex).

2.2. Plant material, kernel obtaining and grinding

T. catappa nuts were obtained from fruits harvested in August 2013 in Calavi (South of Benin) and a voucher

specimen (AA6627/HNB) was conserved at the University of Abomey-Calavi Herbarium. Kernels were obtained by manually crushing nuts dried in the sun for a week. After drying in an oven at 80 °C for 48 h, they were skinned, milled in a domestic electric coffee-grinder (Moulinex KM1, type 27-2761743-85) and then sieved to obtain a fine flour useful for extraction and analysis. The whitish flour obtained was stored in self-sealing polyethylene bags at 7 °C until further use.

2.3. Oil extraction and defatted kernel preparation

T. catappa kernel flour was extracted with hexane in a Soxhlet apparatus, by the method described by Kpoviessi et al. [16], to give the oil. The extraction was carried out in triplicate. The defatted kernel was the dried kernel residue of this extraction.

2.4. Kernel flour analysis methods

Moisture and ash were determined by AACC standard methods 44-15A and 08-01, respectively (AACC1984), protein and total carbohydrate, respectively, according to Biuret [17] and phenol sulfuric [18] methods by using a Jenway Genova spectrophotometer and the energy content value (kcal) was estimated using recommended coefficients used for the analysis of vegetable material [19]. The Kjeldahl method was also used to determine the protein content of the defatted kernels [20]. Phytochemical screening of kernels and defatted kernels was performed according to the standard procedures: Mayer's and Dragendorff's tests for alkaloids, *Fehling's* test for free reducing sugars, *Fehling's* test for glycosides, Liebermann–Burchard's test for triterpenoids and steroids, foam test for saponins, Shinoda's and sodium hydroxide tests for flavonoids, ferric chloride test for tannins, Guignard's test for free cyanogenetics derivatives and Borntrager's test for free anthraquinones were carried out [21]. Oil content determination of the kernel was carried out by extraction with hexane in a Soxhlet apparatus according to standard NF V03-924. The content and isolation of the unsaponifiable matter were obtained by the method described by Kpoviessi et al. [16] including saponification of oil in KOH/ethanol (2N) at reflux for 2 h, extraction of unsaponifiable matter with hexane, washing of the organic phase with NaOH/H₂O (3%), removing of trace of water, filtration, drying and determination of weight on a Mettler Toledo Balance. Total phenolics, tannins and phenolics not retained by the hide powder CRS were determined by using the Folin–Ciocalteu reagent method described in the European pharmacopoeia [22] and were monitored with a double beam UV/VIS spectrophotometer UVIKON 933 (Kontron Instruments, Milan, ITALY).

For mineral analysis, the acid digestion method was used [23]. Mineral elements were measured after calcination at 450 °C followed by acid digestion. A volume of 2 mL of HNO₃ (70%) and 1 mL of HF (48%) was added into the ignition residue. Then, the mixture was heated and evaporated to dryness. One millilitre HClO₄ was added to the residue and the mixture was evaporated again to dryness. The residue was dissolved in 2 mL of mixture of HCl:HNO₃,

3:1, v/v, and diluted with distilled water up to 50 mL in total. The contents of minerals were determined by inductively coupled plasma optical emission spectrometry (ICP-OES) on a Varian Vista-MPX (Japan) type spectrometer equipped with a Charge Coupled Device (CCD) detector. All these analysis are carried out at least in triplicate.

2.5. Oil analysis methods

2.5.1. Physico-chemical analysis

Density, acid (IA), saponification (IS), iodine (II) and peroxide values (PVs) were respectively determined according to the NFT 60-214 standard, NFT60-204, NFT 60-206, NF ISO 3961 and NFT 60-220. The ester value (IE) was calculated on the basis of analytical data using the formula: IE = IS – IA. The calorific value was calculated using the approximate formula of Batel et al. [24]: CV (kJ/kg) = 47645 – 4.187 × II – 38.31 × IS. Values of all these analysis are expressed as mean ± standard deviation (*n* = 3).

2.5.2. GC-FID determination of the fatty acid profile

Fatty acid profiles were obtained by gas–liquid chromatography of the fatty acid methyl ester derivatives. Fatty acids from the oil extract were methylated in a solution of KOH in methanol (0.1 mol/L) at 70 °C for 60 min, then in a solution of HCl in methanol (1.2 mol/L) at 70 °C for 20 min, and finally extracted with hexane. Fatty acid methyl esters (FAMES) were separated and quantified with a gas–liquid chromatograph (GC Trace ThermoQuest, Milan, Italy) equipped with a flame ionization detector, an automatic injector and a fused silica capillary column (100 m × 0.25 mm internal diameter) coated with a 0.2 μm film of biscyanopropylpolysiloxane (Rt-2560, Restek, Bellefonte, PA, USA). The system used H₂ as the carrier gas and operated at a constant pressure of 200 kPa. Splitless injection mode was used minimizing the risk of discrimination between FAs with very different volatilities. The initial oven temperature was 80 °C; it increased at 25 °C/min to 175 °C (held for 25 min), then increased at 10 °C/min to 205 °C (held for 4 min), then increased at 10 °C/min to 225 °C (held for 20 min) and finally decreased at 20 °C/min to 80 °C. The temperature of the flame ionization detector was maintained at 255 °C. Hydrogen flow to the detector was 35 mL/min and air flow was 350 mL/min. A calibration mixture of fatty acid standards was processed in parallel. The data were analyzed by using the Chromquest 3.0 software. Each peak was identified and quantified by comparison of retention times with pure FAME standards. Fatty acids are expressed as the percent of total fatty acids quantified within an individual sample. A total of forty pure FAME standards were used.

2.5.3. Antioxidant activity

For the evaluation of the antioxidant activity, the ranges of concentrations of oils (final concentration ranges: 1536–1.5 μg/mL) are prepared in methanol with tween 80 as the surfactant. According to a modified method of Mensor et al. [25] and Lee Mei Ling et al. [26], 1 mL from 0.3 mM methanol solution of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) was added into 2.5 mL sample or standards. The solution was mixed vigorously and left to stand at room temperature for 30 min in the dark. For each concentration,

the test is repeated three times. The absorbance of the mixture was measured at 518 nm using a Genesis 6 model spectrophotometer (CAT 335908-02 SN 2M8F349001, Japan) and the free-radical scavenging activity was calculated as follows:

Scavenging effect (%)

$$= [1 - \{\text{absorbance of sample/absorbance of control}\}] \times 100$$

The scavenging percentage of all samples was plotted. The final result was expressed as an EC₅₀ value (the concentration of sample producing 50% scavenging of the DPPH radical; mg/ml) and the antiradical power (ARP equal to 1/EC₅₀) was calculated [27].

DPPH solution in methanol with the surfactant was used as the negative control and ascorbic acid [28] solution as the positive control. Their absorbance is measured under the same conditions as the test samples.

2.6. LC-MS analysis of unsaponifiable matter and identification of sterols

Acetonitrile LC/MS grade and methanol LC/MS grade were purchased from Carl Roth (Karlsruhe, Germany). Individual standard solutions (17 sterol standards from Sigma Aldrich (Steinheim Germany), Acros Organics (New Jersey USA) and Fluka Chemie (Buchs Switzerland)) were prepared by dissolving 1 mg of each compound in 1 mL of methanol.

All HPLC-MS analyses were carried out on an Accela system coupled to a LTQ-Orbitrap XL hybrid mass spectrometer equipped with an APCI source (Thermo Fisher Scientific, Bremen, Germany). Chromatographic separations were performed on a Phenomenex Gemini C18 Prevail column (150 × 2.00 mm i.d., 3 μm) using a linear gradient from 85% methanol-15% water (1% acetonitrile) to 100% methanol in 15 min and maintained at 100% methanol for 20 min. The flow rate was 0.2 mL/min, the injection volume was 10 μL and the column temperature was kept at 30 °C. PDA spectra were recorded from 190 to 600 nm.

For HRMS experiments, data were acquired in positive ion mode using full-scan MS with a mass range of 100–2000 *m/z*. The orbitrap operated at 30,000 resolution (FWHM definition). All experimental data were acquired using daily external calibration prior to data acquisition.

Table 1

Physico-chemical composition of *T. catappa* kernels expressed on a dry weight (DW) basis and per 100 g (flour). The contribution to the recommended daily intake (RDI) is expressed in percentage (% CS) (mean ± sd, *n* = 3).

| Constituents | Per 100 g of DW | RDI ^a | % CS | Congo ^d (Per 100 g of DW) |
|------------------------------------|---------------------|------------------------|--------------|--------------------------------------|
| Moisture (g) | 5.50 ± 0.88 | | | 4.13 ± 0.24 |
| Oil content (g) | 61.76 ± 1.01 | 44–97 ^a | 63.67–140.36 | 51.80 ± 0.21 |
| Proteins (g) | 20.14 ± 0.95 | 56 ^a | 35.96 | 23.78 ± 0.15 |
| Sugars (g) | 7.81 ± 0.09 | 130 ^a | 6.01 | 16.02 |
| Ash content (g) | 3.98 ± 0.43 | | | 4.27 ± 0.74 |
| Energy content (kcal) ^b | 593.95 ^c | 2000–2500 ^a | 23.75–29.69 | 548.78 |

^a Dietary Reference Intakes for energy, carbohydrate, fat, fatty acids, cholesterol and protein considering a body weight of 70 kg [52].

^b Energy content calculated on the basis of composition, considering the following values: protein (2.44 kcal/g), sugar (3.57 kcal/g), lipid (8.37 kcal/g) [19].

^c Contribution (%) of lipid to energy content ($61.76 \times 8.37 \times 100/593.95 = 87.03$) [19].

^d Matos et al., 2009 [8].

The following APCI inlet conditions were applied : vaporizer temperature, 450 °C; sheath gas (N₂) flow rate, 25 a.u. (arbitrary unit); auxiliary gas (N₂) flow rate, 25 a.u.; sweep gas (N₂) flow rate, 5 a.u.; discharge current, 5 μA; capillary temperature, 250 °C; capillary voltage, 46 V; tube lens, 55 V.

Collision induced dissociation (CID) was recorded at a relative collision energy of 25%. Data processing was performed with Excalibur software (version 1.1). Selectivity, described in a previous study [29], was obtained by detection in the MS² mode, which eliminated possible interference with compounds of the same retention time in LC and allowed identifications [30].

2.7. Statistical analysis

Student's *t*-test was used to test the significance of differences between results obtained for different samples, and between results for samples and controls (GraphPad Prism 4.0; GraphPad Software Inc., San Diego, USA). The statistical significance was set at *P* < 0.05 [31,32].

3. Results and discussion

3.1. Nutritional properties of *T. catappa* kernels

The nutritional properties of *T. catappa* kernels are summarized in Table 1. The results obtained show that they had 5.50% (w/w) of moisture, lower than the 10% recommended by codex alimentarius [33], 61.76% of oil, 20.14% of protein, 7.81% of total sugars and 3.98% of total ash.

The kernels contained a high level of lipids that represent about 87% (Table 1) of their caloric content providing 63–140% of the RDI of lipids for adults, per 100 g. The level of lipids (extracted by hexane 61.76%) was similar to those obtained from Somalia (56.5%) [13], Congo (51.80%) [8,34], Ghana (52.11%) [10] and Brazil (49%) [15] (extracted by ether) (Table 2). According to Guillermo-Arrazola et al. [35] changes in levels of lipids could be due to different factors such as a variety of plants, climate, place, period of harvest and extraction methods. The level of *T. catappa* kernel oil was more than those of most conventional oils such as soybean (14%), palm fruit (20%), palm kernel (36%), groundnut (42%) [36] cotton (35%) [37] and unconventional oils such as *Jatropha curcas* L. (50%), *Sesamun indicum* L. (50%) [16], *Mangifera indica* L. (12%) [38] and *Ceratotheca sesamoides*

Table 2

Oil and protein contents (%) of *T. catappa* kernels from different countries compared to those of some conventional and unconventional kernels or seeds.

| Kernels or seeds | Oil content (%) | Proteins (%) | |
|-------------------|-------------------------------------|-----------------|--------------------|
| <i>T. catappa</i> | Benin | 62 | |
| | Somalia | 57 ^a | — |
| | Congo | 52 ^b | 24 ^b |
| | Nigeria | 57 ^b | 22–32 ^k |
| | Ghana | 52 ^c | — |
| | Brazil | 49 ^d | — |
| Conventional | Soybean | 14 ^e | 40 ^l |
| | Palm fruit | 20 ^e | — |
| | Groundnut | 42 ^e | 48 ^j |
| | Palm kernel | 36 ^e | 10 ^l |
| | Cotton | 35 ^f | 22 ^m |
| Unconventional | <i>Jatropha curcas</i> L | 50 ^g | 33 ⁿ |
| | <i>Sesamun indicum</i> L | 50 ^g | 18–25 ^o |
| | <i>Mangifera indica</i> L | 12 ^h | 6 ^p |
| | <i>Ceratotherca sesamoides</i> Endl | 26 ⁱ | 21–22 ^q |

- a [13].
 b [8,34].
 c [10].
 d [15].
 e [36].
 f [37].
 g [16].
 h [38].
 i [39].
 j [33].
 k [3,5,7].
 l [53].
 m [54].
 n [55].
 o [56].
 p [57].
 q [58].

Endl. (26%) [39] (Table 2). *T. catappa* kernels appeared as a relatively good source of dietary proteins, 100 g of their flour contributing to more than 35% of the RDI (Table 1). This protein content (20.14%) was close to that described in Congo kernels (23.78%) [8], less than that (22–32%) reported by Olatidoye et al. [3], Mbah et al. [5], and Oderinde and Ajayi [7] for Nigeria and that of some current oilseeds such as groundnut (48%), sunflower (34%) and soy (40%) [33] (Table 2) but higher than that of the majority of our daily food cereals (maize, sorghum, millet, rice, etc), which generally does not exceed 13%. The sugar content (7.81%) was much lower than that obtained by Olatidoye et al. (49.9%) [3] and 2 fold lower than that obtained by Matos et al. [8] in Congo kernels. Proteins and sugars contribute to more than 10% (12.96%) of the total bio-available energy of the kernels which are good sources of energy (Table 1). They contribute to more than 23–30% of the RDI and their energy value was slightly more than that found from Congo [8].

The level of ash represented 3.98% of the kernel's DW (Table 1). It was similar to those obtained by N'zikou et al [34].

Mineral analysis of defatted kernels (100 g) of *T. catappa* (Table 3) showed that phosphorus was the most abundant (1804 mg/100 g) among the macro-elements, followed by potassium (1718 mg/100 g) and magnesium (729.09 mg/100 g). This contributed for more than 36% of recommended daily intake of potassium, about 41% for calcium

Table 3

Protein and mineral compositions of *T. catappa* defatted kernels expressed on a dry weight (DW) basis and per 100 g (flour). The contribution to the recommended daily intake (RDI) is expressed in percentage (% CS).

| Minerals (mg) | 100 g DW | RDI/Al ^f | % CS |
|---------------|----------|---------------------------|---------------|
| P (mg) | 1804 | 700 ^c | 257.71 |
| K (mg) | 1718.12 | 4700 ^d (Al) | 36.55 |
| Mg (mg) | 729.09 | 310–420 ^b | 173.59–235.19 |
| Na (mg) | 37.60 | 1500 ^d (Al) | 2.50 |
| Ca (mg) | 415.01 | 1000 ^b (Al) | 41.50 |
| Fe (mg) | 16.15 | 8–18 ^b | 89.72–201.87 |
| Zn (mg) | 9.67 | 8.0–11 ^e | 87.90–120.87 |
| Mn (mg/100 g) | 4.25 | 1.8–2.3 ^e (Al) | 184.78–236.11 |
| Cu (mg) | 4.63 | 0.90 ^e | 514.44 |
| Proteins (g) | 55.3 | 56 ^a | 98.75 |

^a Dietary Reference Intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol and protein considering a body weight of 70 kg [52].

^b WHO/FAO (2004) [51].

^c Dietary Reference Intakes for calcium, phosphorus, magnesium, vitamin D, and fluoride [52].

^d Dietary Reference Intakes for water, potassium, sodium, chloride, and sulfate [52].

^e Dietary Reference Intakes for vitamin A, vitamin K, arsenic, boron, copper, iodine, iron, manganese and zinc [52].

^f Al – adequate intake.

and much more than 100% of recommended daily intake of phosphorus and magnesium for a body weight of 70 kg.

Regarding the microelements, the quantitative data for the defatted kernels indicated a large amount of iron (16.15 mg/100 g), followed by zinc (9.67 mg/100 g), copper (4.63 mg/100 g) and manganese (4.25 mg/100 g). As iron, copper and zinc are constituents of various important proteins and enzymes involved in macronutrient metabolism and body function [40], their high contents could contribute to explain the use of the mature fruits in folk medicine [41].

Considering the recommended dietary intake guidelines; 100 g of the defatted kernels covers much more than 100% of the requirements in manganese and copper. They also contained 55.3% of proteins that contributed to more than 98% of the RDI (Table 3). This level of proteins was slightly higher than that (49.25%) obtained by Beri et al. [6] in the defatted kernels from India and Oderinde et al. (43.18%) [7] for defatted kernels from Nigeria. The difference between this level (55.3%) and that obtained in the kernel (20.14%) is explained by the high level of oil (61.76% w/w) removed.

Phytochemical analysis on kernels and defatted kernels showed that they contained (Table 4) alkaloids, catechic and gallic tannins, quinone derivatives, mucilage, reducing compounds, combined anthracene derivatives and steroids which may possess several biological properties [42]. Quantification using the Folin–Ciocalteu reagent method helped to determine the content of total phenolics (3.55 ± 0.10%), phenolics not retained by the hide powder CRS (3.00 ± 0.03%), and tannins (0.56 ± 0.07%) in the kernel (Table 4). The presence of these compounds could explain in part the use of this kernel in folk medicine [9,10].

3.2. Fatty acid composition of *T. catappa* oil

Analysis of the fatty acid profile of *T. catappa* oil showed the presence of a total of 17 fatty acids (Table 5). Unsaturated

Table 4

Phytochemical screening of *T. catappa* kernels and defatted kernels. Total phenolic, polyphenols and tannins contents are expressed in g PE/100 g dry weight (DW) of kernels (mean \pm sd. $n = 3$).

| Chemical groups | Kernels | Defatted kernels |
|---------------------------------|-------------------|------------------|
| Alkaloids | + | + |
| Tannins | ++ | ++ |
| Catechic tannins | ++ | +++ |
| Gallic tannins | + | ++ |
| Flavonoids | – | – |
| Anthocyanins | – | – |
| Leucoanthocyanins | + | ++ |
| Quinone derivatives | ++ | ++ |
| Saponosides | – | – |
| Triterpenoids | – | – |
| Steroids | ++ | ++ |
| Cyanogenic derivatives | – | – |
| Mucilage | ++ | ++ |
| Reducing compounds | ++ | ++ |
| Coumarins | – | – |
| Free anthracene derivatives | + | – |
| Combined anthracene derivatives | + | + |
| | g/100 g DW | |
| Total phenolics (g PE) | 3.55 \pm 0.10 | |
| Polyphenols (g PE) | 3.00 \pm 0.03 | |
| Tannins (g PE) | 0.56 \pm 0.07 | |

(–) absent; (+) present; (++) abundant; (+++) most abundant.

Table 5

Fatty acid composition of the *T. catappa* kernel, expressed in % of total fatty acids (%TFA) and in g per 100 g of meal and the recommended daily intake (RDI).

| Fatty acid | % TFA | g/100g | RDI |
|----------------------------------|--------|--------|----------------------------|
| Caproic acid (C6:0) | 0.08 | 0.05 | |
| Capric acid (C10:0) | 0.03 | 0.02 | |
| Lauric acid (C12:0) | 0.16 | 0.10 | |
| Myristic acid (C14:0) | 0.14 | 0.09 | |
| Pentadecylic acid (C15:0) | 0.02 | 0.02 | |
| Palmitic acid (C16:0) | 40.03 | 24.72 | |
| Margaric acid (C17:0) | 0.12 | 0.07 | |
| Stearic acid (C18:0) | 4.49 | 2.78 | |
| Arachidic acid (C20:0) | 0.52 | 0.33 | |
| Palmitoleic acid (C16:1, C9) | 0.39 | 0.25 | |
| Oleic acid (C18:1, C9) | 26.19 | 16.17 | |
| Vaccenic acid (C18:1, C11) | 0.82 | 0.51 | |
| Linoleic acid (C18:2, c9c12) | 26.64 | 16.45 | 11–17 g/day ^a |
| Linoleic acid (C18:2, t9t12) | 0.00 | 0.00 | |
| Linolenic acid (C18:3, C9C12C15) | 0.09 | 0.06 | 1.1–1.6 g/day ^a |
| Gadoleic acid (C20:1, c11) | 0.08 | 0.05 | |
| Behenic acid (C22:0) | 0.12 | 0.08 | |
| SFA/UFA | 0.84 | | <1 ^b |
| MUFA/PUFA | 1.03 | | |
| ω -6/ ω -3 | 269.09 | | 4 ^c |

SFA: saturated fatty acid; UFA: unsaturated fatty acid; MUFA: mono unsaturated fatty acid; PUFA: poly unsaturated fatty acid.

^a Dietary Reference Intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids [52].

^b FAO (2008) [43].

^c Schaefer (2002) [44].

fatty acids represent 54.23% of total fatty acids and this value was close to that of saturated fatty acids; palmitic (C16:0; 40.03%), oleic (C18:1; 27.08%), linoleic (C18:2; 26.64%), and stearic acids (C18:0; 4.49%) being the major components (Table 5). The saturated to unsaturated fatty acid ratio (SFA/UFA) obtained in the present study (0.84) is close to the dietary recommendations [43] but slightly higher than the

reported values [13,15,34]. The monounsaturated to polyunsaturated fatty acid ratio (MUFA/PUFA) was low (1.01), whereas the Ω -6 to Ω -3 ratio was much higher than the maximum recommended value of 4 [44]. The high level of linoleic acid, which is one of the most important polyunsaturated fatty acids in human food, makes this oil consumption beneficial for health. It was reported that this fatty acid contributes to heart vascular disease prevention [45]. The content of fatty acids was similar to those mentioned in Congo and Porto Rico kernel oils [8,14]. The fatty acid profile of this oil was close to that of palm oil [35].

3.3. Physico-chemical characteristics of *T. catappa* kernel oil

The oil was liquid at room temperature with a yellow color and a pleasant smell of roasted almonds. The characteristics of oil are listed in Table 6.

The acid value of the oil (2.24 ± 0.01 mg KOH/g) was in accordance with the standard values (≤ 4 mg KOH/g) set by the Codex Alimentarius [33] for crude edible oils and close to those (2.42–3.02) reported by Matos et al. [8] for this oil from two localities of Congo and by Olaidoye et al. (1.3) [3] for Nigeria, but less than that (7.39) obtained by Asenjo et al. [14] from Porto Rico. This oil had a low free fatty acid level and then would possess according to this point, a proven nutritional quality for food use.

The saponification value (175.33 mg KOH/g) was higher than 128 mg KOH/g obtained by Olatidoye et al. [3] and slightly less than those (187.6 and 196 to 207) obtained, respectively, by Asenjo et al. [14], and Matos et al. [8].

The oil iodine value (74.48 g of I₂/100 g) was close to the reported values [8] which are slightly less than those of Dos Santos et al. [15] and conventional food oils such as peanut oil (86–105 g I₂/100 g). The value found was higher than 65 g I₂/100 g mentioned by Olatidoye et al. [3] and those of palm oil 50–55 g I₂/100 g [46] indicating that this oil may be classified as an unsaturated one.

The peroxide value (3.71 mequiv O₂/kg) was lower than the maximum value (15 mequiv O₂/kg) prescribed for the raw food oils [33], but in accordance with the previous value (2.8 mequiv O₂/kg, [3]) and less than the minimum value (10 mequiv O₂/kg) specified for the rancid oils [47,48] demonstrating the good quality of this oil.

The calorific value of the oil (40616.78 kJ/kg) was greater than 35000 kJ/kg (Table 6). This oil could be used as a biofuel and as motor lubricant [49].

3.4. Sterols and triterpenes from unsaponifiable matter of *T. catappa* oil

The unsaponifiable matter content of *T. catappa* oil was $1.76 \pm 0.32\%$. This content was higher than those (0.65%) obtained by Asenjo et al. [14]. LC/MS analysis allowed identification of six sterols and triterpenes: santonin, lupeol, cholesterol, campestanol, beta-sitosterol and sitosterol (Table 7). These components identified for the first time in this kernel oil can be linked to certain pharmacologic properties (body weight loss, total cholesterol reduction, antidiabetic, and heart disease prevention) of *T. catappa* kernel and can explain in part its use in folk medicine [50].

Table 6Physico-chemical characteristics of *T. catappa* kernel oil compared to groundnut and palm oils (mean \pm sd. $n = 3$).

| Characteristics | <i>T. catappa</i> oil | | | Groundnut oils ^b | Palm oils ^d |
|-----------------------------------------------------|-----------------------|----------------------|---------------------|-----------------------------|------------------------|
| | Benin | Nigeria ^a | Congo ^c | | |
| Acid value (mg KOH/g) | 2.24 \pm 0.01 | 1.3 | 2.42–3.02 | <4 | <4 |
| Saponification value (mg de KOH/g) | 175.33 \pm 0.42 | 128 | 196–207 | 187–196 | 190–209 |
| Calculated ester value | 173.09 | nd | nd | nd | nd |
| Peroxyde value (mequiv O ₂ /kg) | 3.71 \pm 1.2 | 2.8 | 0.41–0.51 | <15 | <15 |
| Iodine value (g d ¹ ₂ /100 g) | 74.48 \pm 0.65 | 65 | 80.89–82.43 | 86–107 | 50–55 |
| Calorific value (kJ/kg) | 40,616.78 | 42496 | 39,798.12–39,370.44 | 39,688.98–40,121.55 | 39,408.31–40,157.1 |

nd = not determined.

^a Values obtained on *T. catappa* oil from Nigeria [3].^b Values obtained on groundnut oils taken from reference [36].^c Values obtained on *T. catappa* oil from Congo [8].^d Values obtained on *Palm* oil taken from reference [46].**Table 7**LC-MS identification of sterols and triterpenes in unsaponifiable matter of *T. catappa* kernel oil.

| No | RT | Exact mass | Quasimolecular ion mass | | Mass formula | Molecular name |
|----|-------|------------|-------------------------|----------------------|------------------------------------------------|-----------------|
| | | | M+H | M+H–H ₂ O | | |
| 1 | 2.65 | 246.13 | 247.13 | | C ₁₅ H ₁₈ O ₃ | Santonin |
| 2 | 29.03 | 426.39 | 427.39 | 409.38 | C ₃₀ H ₅₀ O | Lupeol |
| 3 | 29.27 | 386.35 | | 369.35 | C ₂₇ H ₄₆ O | Cholesterol |
| 4 | 34.40 | 402.39 | | 385.38 | C ₂₈ H ₅₀ O | Campestanol |
| 5 | 34.69 | 414.39 | 415.39 | 397.38 | C ₂₉ H ₅₀ O | Beta-sitosterol |
| 6 | 37.36 | 416.4 | | 399.4 | C ₂₉ H ₅₂ O | Sitostanol |

Table 8Antioxidant activity (EC₅₀ expressed in mg/mL) and antiradical power (ARP) of *T. catappa* kernel oil (mean \pm sd. $n = 3$).

| Sample | Antioxidant activity (EC ₅₀ , mg/mL) mean \pm standard deviation | Antiradical power ARP (ARP = 1/EC ₅₀) |
|-----------------------|----------------------------------------------------------------------------------|------------------------------------------------------|
| <i>T. catappa</i> oil | 6.61 \pm 0.70 ^a | 0.15 |
| *Ascorbic acid | 0.02 \pm 0.00 ^b | 50 |

EC₅₀ = concentration of the sample producing 50% scavenging of the DPPH radical.

ARP = Antiradical power.

Data in the same column followed by different letters (^{a,b,c,...}) are statistically different from those of Student's *t*-test ($P < 0.05$).Values are means \pm standard deviation of three different experiments.

*Positive control.

3.5. Antioxidant activity of *T. catappa* kernel oil

The antioxidant activity of the crude oil was determined and expressed as EC₅₀ (Table 8). Its value was 6.61 mg/mL and the antiradical power was 0.15. This oil had a low capacity of neutralizing the DPPH free radical, since its antiradical power is at least 50 times lower than that of ascorbic acid when used as a positive control, but is not devoid of antioxidant activity.

4. Conclusion

This is the first report on the detailed chemical properties including nutritional composition, phenolic, phytosterol and triterpene content, phytochemical screening and antioxidant capacity of *T. catappa* crude or defatted kernels from Benin. The results showed that these kernels possess interesting nutritional properties and compounds with possible pharmacological activities as triterpenes and phytosterols. They

contained 61.76% of unsaturated edible oil much more than most conventional oils. Physico-chemical properties of the oil were in accordance with Codex Alimentarius recommendations. It was rich in palmitic (40.03%), oleic (27.08%) and linoleic acids (26.64%). This unsaturated oil classified in the oleic-linoleic acid group possessed weak antioxidant activity and can be used in food and as a technical adjuvant (ointment, soap, biofuel, paint, lubricants, insecticides, etc.).

T. catappa defatted kernels contained interesting amounts of minerals, proteins (55.3%) and chemical compounds such as alkaloids, quinone derivatives, gallic and catechic tannins, free anthracene derivatives and reducing compounds. They conserved a good nutritional composition and some therapeutic compounds and could play a role in disease treatment.

Benin kernels, very appreciated by children, possess good nutritional properties similar to groundnut and *Cesamum indicum* seeds. They particularly showed a high level of oil with palmitic acid as the most important fatty acid, with a composition close to those of *J. curcas* and palm oil. They also contain high levels of minerals and proteins and could be useful in children feeding.

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