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# Chemical-functional composition of *Terminalia catappa* oils from different varieties

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**SUMMARY:** This study aimed to extract and physical-chemically characterize *Terminalia catappa* L. kernel oil from purple (CR) and yellow (CA) varieties. Physical-chemical parameters, composition of fatty acids, nutritional quality indices, bioactive compounds and antioxidant capacity of both oil varieties were evaluated according to the literature. Both oils presented low levels of acidity and peroxides, besides the predominance of unsaturated fatty acids, ~63% of oleic and ~26% of linoleic acids, which influenced its nutritional indices. The CR oil variety exhibited a higher content in anthocyanin (18.3 ± 1.5 mg·100 $g^{-1}$), ascorbic acid (68.4 ± 2.02 mg·100 $g^{-1}$) and total polyphenol contents (152.3 ± 2.4 mg GAE·$g^{-1}$), and a good antioxidant activity (38.6 ± 2.2 µg TE·$g^{-1}$) determined by TEAC assay, when compared to the CA oil ($p < 0.05$). Therefore, the results confirm the importance of *T. catappa* as a lipid source for human consumption to be used in the development of food products.

***KEYWORDS:*** *Antioxidant activity; Bioactive substances; Linoleic acid; Oleic acid; Tropical almond; Vegetable oil.*

**RESUMEN:** ***Composición química-funcional de aceites de* Terminalia catappa *de diferentes variedades***. El objetivo de este estudio fue extraer y caracterizar físico-químicamente aceite de semilla de *Terminalia catappa* de las variedades violeta (CR) y amarilla (CA). Se evaluaron parámetros fisicoquímicos, composición de ácidos grasos, índices de calidad nutricional, compuestos bioactivos y capacidad antioxidante de ambas variedades de aceite de acuerdo con a la literatura. Como resultado, ambos aceites presentaron bajos niveles de acidez y peróxidos, y predominio de ácidos grasos insaturados, ~63% de ácido oleico y ~26% de ácido linoleico, lo cual influyó en su perfil nutricional. La variedad de aceite CR presentó un mayor contenido de antocianina (18,3 ± 1,5 mg·100 $g^{-1}$), ácido ascórbico (68,4 ± 2,02 mg·100 $g^{-1}$) y contenido total de polifenoles (152,3 ± 2,4 mg GAE·$g^{-1}$), y una alta actividad antioxidante (38,6 ± 2,2 µg TE·$g^{-1}$) determinado por ensayo TEAC, en comparación con el aceite CA ($p<0.05$). En conclusión, los resultados presentados refuerzan la importancia de *T. catappa* como fuente de lípidos para la ingesta humana y para su uso en el desarrollo de productos alimenticios.

***PALABRAS CLAVE:*** *Aceite vegetal; Ácido linoleico; Ácido oleico; Actividad antioxidante; Almendra tropical; Sustancias bioactivas.*

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## 1. INTRODUCTION

Among the diversity of fruit species in Brazil there are underexploited species that can be used for human nutrition, as well as for the extraction and isolation of functional/bioactive compounds, which can play an important role in maintaining human health. In this context, a native species from tropical and subtropical regions, *Terminalia catappa* L., stands out as an innovative source of fruits and their derivatives. It belongs to *Combretaceae* family, and produces glabrous, rounded and flattened drupaceous fruits. These fruits are commonly named Sea-almond, Tropical-almond, Indian-almond or Malabar-almond (Abdulkadir, 2015).

The Tropical almond fruits initially exhibit a green color, which during the maturation process becomes red-purple and, also may turn yellow (Salawu *et al.,* 2018). The fruits measure 5-7 cm long and 3-6 cm in width, have an exocarp (bark) adhered to an edible fibrous pulp (mesocarp), and a single rigid seed. The fruit pulp is a good source of carbohydrates, up to 76%, and low in lipids, less than 3% (Ladele *et al*., 2016). The seed has an oily endocarp (kernel), containing up to 52% lipids, 38% proteins and minerals, which is covered by a thin peel (Agu *et al*., 2019; Abdulkadir, 2015; Souza *et al*., 2016). Both pulp and seeds are edible (Ladele *et al*., 2016). The lipids extracted from the kernel almond have great potential for application as an edible vegetable oil due to their elevated lipid yield, up to 60%, which has higher value when compared to main commercial oilseeds, such as soybeans, palm, and peanuts (Ladele *et al*., 2016; Jokić *et al*., 2015).

*Terminalia catappa* can be considered a fruit tree with high economic potential because its fruiting starts at around 3 to 5 years of age, with two harvests per year, producing up to 30 kg fruits per year, reaching an estimated world production of more than 700,000 tons in 2004 (Agu *et al*., 2019; Singh and Choudhary, 2012). However, its productivity needs further assessment. In addition, this species is commonly cultivated in several countries as an ornamental tree, a fruit tree and as a vegetable oil source for different applications (Agu *et al*., 2019; Janporn *et al*., 2015; Menkiti *et al.*, 2015). The Tropical almond cultivation generally needs low maintenance, since it has a simple propagation from seeds and can grow quickly in different soils and environments (Ladele *et al*., 2016).

Investigations have been carried out to characterize the Tropical almond fruit and to determine and quantify its bioactive compounds as a source of natural antioxidants (Abdulkadir, 2015; Huang *et al*., 2018). These bioactive compounds have been studied for complementary functions and actions of insulin in the treatment of diabetes, to act regulating dietary constituents in human daily intake and as potential anti-inflammatory agents (Ben *et al*., 2019; Huang *et al*., 2018). A recent study has focused on the nutritional and functional properties of the pulp and kernel oils of *Terminalia catappa* L. obtained by supercritical fluids (Santos *et al*., 2021). In another work from Agu *et al*. (2019), the *T. catappa* kernel oil was chemically modified and characterized as a potential replacement for mineral transformer fluid. In a similar application, the oil from *T. catappa* was used by Silva *et al.* (2020b) to synthetize biodiesel (via methyl route).

It is worth mentioning that in the works in the literature, a few of them have identified which variety of the Tropical almond, purple or yellow, was used in their research. Thus, investigations comparing different varieties of Tropical almond fruit can increase knowledge based on its different chemical compositions and bioactive/nutritional constituents, and guide new applications for the food and chemical industries.

The aim of this research was to evaluate the functional chemical composition of *Terminalia catappa* L. kernel oil, and to compare its purple (CR) and yellow (CA) varieties. Fatty acids and triacylglycerol profile, nutritional quality parameters, bioactive compounds, such as anthocyanins, ascorbic acid and polyphenol contents, and antioxidant activity were investigated.

## 2. MATERIALS AND METHODS

### 2.1. Raw material and oil extraction

Fruit seeds of *Terminalia catappa L.* from purple (CR) and yellow (CA) varieties were harvested on the campus of Federal University of Pará (UFPA) in 2020. The fruits were collected in the following geographical coordination: latitude 01º 27 ‘21”S, longitude 48º 30’ 16”W and altitude 10 m. To ensure the proper taxonomic identification of this plant, some parts of it, such as leaves and fertile material, were collected and deposited in the herbarium Professor Normélia Vasconcelos/UFPA under the code MG nº 3791.

### 2.2. Sampling and oil extraction

The fruits of *Terminalia catappa* L. from purple and yellow varieties were washed to remove any physical dirt, gently peeled off, and then the seeds were manually cracked, and their kernels were removed. The kernels were dried at 60 °C for 24 h in an air-circulation oven (model 81-150, New Lab Equipamentos, Piracicaba, SP, Brazil), and milled in a Willey miller (TE-650 model, Tecnal, SP, Brazil). Subsequently, a solid-liquid extraction was carried out in a Soxhlet apparatus, using hexane as solvent according to the methodology of Silva *et al.* (2020b). All analyses were performed in triplicate. The oil yield (%) was calculated according to the Eq. 1.

$$Oil\ yield\ (\%) = \frac{W_{oil}}{WT_g} \times 100 \quad (1)$$

where $W_{oil}$ is the extracted mass of oil (g) and the $WT_g$ is the total mass of seeds (g).

### 2.2. Physical-chemical analysis of *T. catappa* kernel oils

#### *2.2.1. Quality parameters*

The physical-chemical quality parameters of the *Terminalia catappa* L. kernel oils from purple (CR) and yellow (CA) varieties were determined according to the official methods from the American Oil Chemist's Society (AOCS), as follows: acidity, peroxide and saponification values were determined according to AOCS methods Cd 3d-63, Cd 8-53 and Cd 3-25, respectively (AOCS, 2004). The true density (ρ, g $m^{-3}$) was measured using a DA–130 digital density meter (Kem Kyoto Electronics, Japan) at room temperature (~25 °C) and the refractive index was investigated according to the Cc 7-25 method (AOCS, 2004).

#### *2.2.2. Fatty acids profile*

The fatty acid (FAs) profile of *Terminalia catappa L.* oils CR and CA was determined as fatty acid methyl esters (FAMEs) according to the established procedure ISO 5509:2000 reported by the International Organization for Standardization (ISO, 2000). After phase separation, the supernatant was collected for subsequent gas chromatographic analysis with flame ionization detector (GC-FID) (Thermo Scientific Trace GC Ultra) using a wall-coated open-tubular column (WCOT). The analysis was performed in a gas chromatograph (Varian 430 model, Agilent Technologies, CA, USA) equipped with a microcomputer with the software Galaxie Chromatography under the following parameters: fused silica SP®-2560 capillary column (Merck, USP-G5, SUPELCO, USA) of 100 m in length and 0.25 mm internal diameter, containing 0.2 μm of polyethylene glycol. The operation conditions were: 50:1 split injection ratio, column temperature at 140 °C for 5 min programmed with an increasing rate of 4 °C·$min^{-1}$ up to 240 °C, helium as carrier gas in 37 psi isobaric pressure, 20 cm·$sec^{-1}$ linear velocity, make up gas: 29 mL·$min^{-1}$ helium flow, 250 °C injector temperature, autosampler model Varian CP8410, detector temperature 250 °C. The peaks were identified by comparing peak retention time to the known FAMEs standard (37-Component FAME Mix - methyl esters of fatty acids ranging from $C_4$ to $C_{24}$ CRM47885, Supelco). The quantitative composition was carried out by area normalization, and expressed in mass percentage as established by the official method Ce 1-62 (AOCS, 2004). The samples were analyzed in triplicate.

#### *2.2.3. Nutritional quality indices*

The nutritional quality indices in the *Terminalia catappa L* oils from purple and yellow varieties were established based on their respective FAs profiles, which were classified according to the presence and number of double or triple bonds: saturated fatty acids (SFA), unsaturated fatty acids (UFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). The following indices were used to investigate its nutritional quality: atherogenicity ($AI$) and thrombogenicity indices ($TI$) were determined according to Ulbricht, Southgate (1991), and calculated according to Eq. 2 and Eq. 3, respectively. The hypocholesterolemic/hypercholesterolemic ratio ($h/H$) was determined as defined by Santos-Silva *et al.* (2002) and calculated using the Eq. 4. The calculated oxidative stability value ($COX$) was defined according to Silva *et al.* (2020a) as expressed in the Eq. 5.

$$AI = \frac{[(C_{12:0} + (4 \times C_{14:0}) + C_{16:0})]}{(PUFA + MUFA)} \quad (2)$$

$$TI = \frac{(C_{12:0} + C_{16:0} + C_{18:0})}{[\,(0.5 \times MUFA) + (0.5 \times n6 - PUFA) + (3 \times n3 - PUFA) + (\frac{n3 - PUFA}{n6 - PUFA})]} \quad (3)$$

$$h/H = \frac{C_{18:1} + PUFA}{(C_{14:0} + C_{16:0})} \quad (4)$$

$$COX = \frac{(C_{18:1}) + (10.3 \times C_{18:2}) + (21.6 \times C_{18:3})}{100} \quad (5)$$

### *2.2.4. Triacylglycerol composition*

The triacylglycerol composition in the *Terminalia catappa L.* oils from purple and yellow varieties was estimated based on the 1,3-random-2-random distribution hypothesis using the software PrOleos®, which predicts the molar percentage of triacylglycerols present in oil based on its fatty acid composition (Antoniosi Filho *et al*., 1995). This software is available online at the website "https://lames.quimica.ufg.br/p/4035-apoio-didatico".

### *2.2.5. Analysis of bioactive compounds and antioxidant capacity*

The bioactive compounds and antioxidant activity in *Terminalia catappa* L. kernel oils from purple and yellow varieties were investigated. The bioactive compounds were analyzed as anthocyanin, ascorbic acid and total polyphenol contents, and the antioxidant activity was determined based in the Trolox Equivalent Antioxidant Capacity (TEAC) assay. Prior the analysis, the oil samples were solubilized in isopropyl alcohol at 320 mg·mL$^{-1}$ concentration.

***Anthocyanin content.*** The content of anthocyanins was determined as reported by Silva *et al*. (2014). About 1 g of each sample was mixed with 10 mL of a 1.5N HCl in 85% ethanol solution. The samples were homogenized and left to rest overnight under refrigeration and dark covered. Then, the absorbance of the samples was measured at 535 nm wavelength using an UV-Vis spectrophotometer (model UV-1800, Shimadzu, Tokyo, Japan). The analysis was performed in triplicate. The anthocyanins' content was calculated using the Eq. 6 and results were expressed as mg·100g$^{-1}$.

$$Anthocyanin\ content = \frac{[Abs \times dilution\ factors] \times 1000}{[W_{sample} \times \varepsilon^{1\%}_{1cm,535}]} \quad (6)$$

where $Abs$ is the measured absorbance of the sample at 535 nm, $W_{sample}$ is the weight of the sample and $\varepsilon^{1\%}_{1cm,535}$ is the absorption coefficient for anthocyanins, which is equal to 982 g·100 mL$^{-1}$cm$^{-1}$.

***Ascorbic acid content.*** The ascorbic acid content was determined by the reduction of the 2,6-dichlorophenol-indophenol compound, according to the adapted methodology of Cunha-Santos *et al*. (2019). About 10 mL of sample were mixed with 2 mL of a 0.03g·mL$^{-1}$ metaphosphoric acid diluted in an acetic acid aqueous solution and titrated with 0.2% 2,6-dichlorophenol-indophenol solution with sodium bicarbonate at 0.21 mg·mL$^{-1}$ concentration, until the appearance of a pink color was persistent for more than 5 s. The 2,6-dichlorophenol-indophenol solution was standardized with an ascorbic acid solution prior to analysis. The sample was analyzed in triplicate and the results were expressed as mg of ascorbic acid per 100 g of sample (mg·100g$^{-1}$).

***Total polyphenol content***. The total polyphenol content of these fractions was analyzed following the Folin-Ciocalteu assay as reported by Aliakbarian *et al*. (2011). Initially, 0.2 mL sample, 4.8 mL deionized water, and 0.5 mL Folin-Ciocalteu reagent (Sigma-Aldrich) were transferred to a 10 mL volumetric flask, and vigorously mixed. Then, 1 mL of a 20% sodium carbonate solution was added, followed by deionized water until reaching a final volume of 10 mL. The solutions were mixed and left to rest at room temperature in the dark for 1 h. An aliquot (~2 mL) of sample was used for the determination of total polyphenols using a UV-Vis spectrophotometer (model UV-1800, Shimadzu, Tokyo, Japan) at a wavelength of 725 nm. Distilled water was considered as blank. The sample was analyzed in triplicate, and the results were calculated based on a standard curve of gallic acid (Sigma-Aldrich) and expressed as mg GAE·g$^{-1}$.

***Antioxidant capacity.*** The determination of the antioxidant capacity from the samples was performed according to the Trolox equivalent antioxidant capacity (TEAC) assay using the ABTS (2,2'-azinobis 3-ethylbenzthiazoline-6-sulfonic acid, from Sigma-Aldrich) reagent as described by Chen *et al*. (2011). The absorbance was measured at 734 nm wavelength using UV-Vis spectrophotometer (model

UV-1800, Shimadzu, Tokyo, Japan). The assay was performed in triplicate against a calibration curve of Trolox (µg Trolox equivalent·$L^{-1}$) and calculated using the following linear equation:

$TEAC = (0.6239 - Absorbance_{734nm})/0.3364$ ($R^2 = 0.997$)

### 2.3. Statistical analyses

The results were statistically analyzed using the Statistica software version 7.0 (Statistica, 2000), by analysis of variance (ANOVA) and Tukey's test at the significance level of 5% ($p < 0.05$).

## 3. RESULTS AND DISCUSSION

### 3.1. Oil extraction

The solid-liquid extraction of the *Terminalia catappa* L. kernel oils from purple (CR) and yellow (CA) varieties showed a lipid yield of 57 and 54%, respectively. These results are higher than the value of 52% found by Silva *et al*. (2020b) and lower than the value of 61.7% obtained by Ladele *et al*. (2016) for kernel oil yield from *Terminalia catappa* seeds. It is worth mentioning that in both studies, hexane was used as solvent for solid-liquid extraction, and the seeds were harvested in Brazil and Benin, respectively.

### 3.2. Physical-chemical analysis of the *T. catappa* kernel oils

#### *3.2.1. Quality parameters*

The results from the quality parameters of the *Terminalia catappa* L. kernel oils from purple (CR) and yellow (CA) varieties are shown in Table 1. The quality parameters in vegetable oils, acidity, and peroxide values, are ruled by the Codex Alimentarius (2001). This institution recommends the maximum values for acidity and peroxide, in crude vegetable oils as 4 mg KOH·$g^{-1}$ and 15 meq·$Kg^{-1}$, respectively.

Table 1. Quality parameters of *Terminalia catappa* kernel oils from purple (CR) and yellow (CA) varieties.

| Quality parameters | Oil samples | |
|---|---|---|
| | CR | CA |
| Acidity value (mg KOH·$g^{-1}$) | 1.25 ± 1.05[a] | 1.55 ± 0.47[a] |
| Peroxide value (meq $O_2$·$Kg^{-1}$) | 2.05 ± 1.17[a] | 3.43 ± 0.72[a] |
| Saponification value (mg KOH·$g^{-1}$) | 185.5 ± 0.18[a] | 180.7 ± 1.05[a] |
| Refractive index | 1.50 ± 0.01[a] | 1.45 ± 0.00[a] |
| Density (g·$m^{-3}$) | 0.91 ± 0.00[a] | 0.90 ± 0.00[a] |

Data represent the mean ± standard deviation of triplicate analyses (n = 3). Different superscript lowercase letters in the same line represent significant differences ($p < 0.05$) at 95% confidence interval according to Tukey's test.

The acidity values for the CR and CA oils are in accordance with the Codex Alimentarius (2001) standard values for crude vegetable oils. When comparing this result to the acidity value for *T. catappa* kernel oil from Benin and Congo, ~2.24 and ~2.42 mg KOH·$g^{-1}$ respectively, from the work of Ladele *et al*. (2016), it was observed that the CR and CA oils presented a lower value. In the work of Janporn *et al*. (2015), the acidity value for the *T. catappa* oil from Thailand was around 2.4 mg KOH·$g^{-1}$, a higher value when compared to the CR and CA oils. The determination of acidity in vegetable oils is an important indicator of the presence of free fatty acids, which can be associated with lipid hydrolytic degradation and quality loss (Ghafoor *et al.*, 2019).

The peroxide values determined for CR and CA oils were below the maximum value recommended by the Codex Alimentarius (2001), demonstrating its good quality. It was observed that the CA oil presented a higher peroxide value than CR, although with no statistically significant difference ($p > 0.5$). When investigating the peroxide value of *T. catappa* oils from Benin, Nigeria and Congo extracted using organic solvents, Ladele *et al.* (2016), found similar values of 3.7, 2.8 and 0.5 meq $O_2$·$Kg^{-1}$, respectively. In another work, the crude oil of *T. catappa* from Thailand presented a lower value for peroxides at 0.65 meq $O_2$·$Kg^{-1}$ (Janporn *et al*., 2015). The peroxide value is a crucial factor in the quality evaluation of edible oils as it can be correlated to the presence of secondary lipid oxidation products and may cause rancidity. Besides, it is well established that high temperatures during processing, storing, as well as long-time exposures to light, humidity and atmospheric oxygen are key factors to lipid oxidation, which is reflected in high levels of acidity and peroxides.

The saponification value for *T. catappa* CR and CA oils was higher than the amount of ~175 mg KOH·$g^{-1}$ obtained by Ladele *et al*. (2016), and ~179 mg KOH·$g^{-1}$ as determined in the work of Janporn *et al*. (2015). When comparing both varieties of *T. catappa* oils, purple and yellow, the first one was found to present a higher saponification value, although

with no significant difference ($p > 0.05$). In the Codex Alimentarius (2001) there is no indication of maximum value for saponification in crude vegetable oils, but there is a recommended value for virgin olive oil of 184 – 196 mg $KOH \cdot g^{-1}$. When considering these limits, the CR and CA oils presented lower values, which is a good indication of quality. The saponification value is commonly used to estimate the average length of FA chains, which may indicate a high percentage of short-chain ester bonds and a higher saponification value.

The physical properties of vegetable oils, such as density, refractive index, viscosity, and other rheological parameters are factors to be considered when considering the processing design of equipment, as well as its proper function, e.g., pumping, settling and filtration (Freitas *et al.*, 2018). Nevertheless, the density and the refractive index of CR and CA are in accordance with the literature (Ghafoor *et al*., 2019; Freitas *et al.*, 2018). For both results, the CR and CA oils presented no significant difference ($p > 0.5$) between each other. Furthermore, the quality parameters of CR and CA oils are in accordance with the literature and international standards (Codex Alimentarius, 2001).

### *3.2.2. Fatty acid profile and nutritional quality indices*

The composition on FAs of *Terminalia catappa* kernel oils from purple (CR) and yellow (CA) varieties is shown in Table 2, and for comparison purposes the FA profiles of authentic vegetable oils from maize, soyabean and palm kernel determined by the Codex Alimentarius (2001) were listed. The FA profiles of CA and CR oils exhibited the predominance of unsaturated fatty acids (UFAs), up to 62.9%, mainly represented by oleic acid. The major proportions of FAs in both samples were oleic acid (up to 39%), follow by palmitic acid (~33%), then linoleic acid (~26%). When comparing the CR and CA oils, the percentages of oleic and linoleic acids presented significant differences ($p < 0.05$), a behavior that was not observed for the other FAs. Furthermore, the FA profiles of CR and CA are in accordance with the literature. In the work of Janporn *et al*. (2015), the oil from *T. catappa*, extracted by solvent using the Soxhlet apparatus, presented a remarkably similar FA profile. For these authors, the major proportions of FAs were oleic acid (~31.7%), followed by palmitic acid (~31.4%), then linoleic acid (~23%).

Table 2. Comparative profiles of fatty acids in *T. catappa* kernel oils from purple (CR) and yellow (CA) varieties and other oilseeds.

| % Total fatty acids | *Terminalia catappa* oils | | Edible vegetable oils* | | |
|---|---|---|---|---|---|
| | CR | CA | Maize | Soyabean | Palm kernel |
| Myristic acid ($C_{14:0}$) | 0.10 ± 0.00 | --- | < 0.3 | < 0.2 | 0.5−1.5 |
| Palmitic acid ($C_{16:0}$) | 31.08 ± 0.00$^a$ | 33.4 ± 0.9$^a$ | 8.6−16.5 | 8−13.5 | 38−43.5 |
| Palmitoleic acid ($C_{16:1}$) | 0.38 ± 0.00$^a$ | 0.29 ± 0.00$^a$ | < 0.5 | < 0.2 | < 0.6 |
| Stearic acid ($C_{18:0}$) | 5.72 ± 0.01$^a$ | 5.62 ± 0.01$^a$ | <3.3 | 2−5.4 | 3.5−5 |
| Oleic acid ($C_{18:1}$ *cis* ω-9) | 39.08 ± 0.02$^a$ | 33.9 ± 2.3$^b$ | 20−42.2 | 17−30 | 39.8−46 |
| Linoleic acid ($C_{18:2}$ *cis* ω-6) | 22.80 ± 0.03$^a$ | 26.0 ± 2.6$^b$ | 34−65.6 | 48−59 | 10−13.5 |
| α-linolenic acid ($C_{18:3}$ ω-3) | 0.06 ± 0.01$^a$ | 0.04 ± 0.05$^a$ | <2 | 4.5−11 | <0.6 |
| Arachidic acid ($C_{20:4}$ ω-6) | 0.62 ± 0.00$^a$ | 0.55 ± 0.01$^a$ | ---- | ---- | ---- |
| Behenic acid ($C_{22:0}$) | 0.19 ± 0.01$^a$ | 0.16 ± 0.06$^a$ | <0.5 | ---- | ---- |
| Σ SFAs | 37.10 | 39.20 | ---- | ---- | ---- |
| Σ UFAs | 62.90 | 60.80 | ---- | ---- | ---- |
| Σ MUFAs | 39.46 | 34.20 | ---- | ---- | ---- |
| Σ PUFAs | 23.50 | 26.70 | ---- | ---- | ---- |
| Σ ω-6 | 23.42 | 26.65 | ---- | ---- | ---- |
| Σ ω-3 | 0.06 | 0.04 | ---- | ---- | ---- |
| Total | 100.00 | 100.00 | ---- | ---- | ---- |

*Values determined from authentic samples by Codex Alimentarius (2001). ---- = Non-defined. Data represent the mean ± standard deviation of triplicate analyses (n = 3). Different superscript lowercase letters in the same line represent significant differences ($p < 0.05$) at 95% confidence interval according to Tukey's test.

The composition of FAs in the *T. catappa* kernel oil from Benin, investigated by Ladele *et al*. (2016), also displayed a similar profile, in which the palmitic (~40%), linoleic (~26.6%) and oleic acids (~26.2%) stood out.

When comparing the FA composition determined by the Codex Alimentarius to the *Terminalia catappa* kernel oils, major differences are found. However, palm kernel oil presented the closest FA profile to the CR and CA oils, mainly due to relatively similar amounts of palmitic and oleic acids, around 40%. Palm kernel oil also presented a significant proportion of linoleic acid, around 13%, which was two times lower than the CR and CA. These results corroborate the edibility of *Terminalia catappa* kernel oils from purple and yellow varieties.

The evaluation of the FA composition of vegetable oils can provide a vital classification of its lipids related to nutritional indices, mainly due to the presence of essential fatty acids, which can be used to correlate it to the prevention of cardiovascular diseases. The nutritional quality indices of *T. catappa* kernel oils from purple (CR) and yellow (CA) varieties are presented in Table 3. For comparison purposes the indices from other tropical fruit oilseeds, *Caryocar villosum*, *Bactris gasipaes*, and *Oenocarpus bacaba*, are displayed in the same table.

The ratio between polyunsaturated and saturated acids (P/S) is a relevant index of the nutritional quality of oils intended for human consumption, as a higher proportion of PUFAs may prevent the increase in body weight in high-fat diets. Nutritional regulations suggest a P/S ratio above 0.4, although this index cannot be taken into account alone for a healthy diet (Domínguez *et al*., 2016). the P/S value determined for the CR and CA oils were inferior to the value determined by Ladele *et al*. (2016), 0.84, and by Janporn *et al*. (2015), 1.4. However, when considering the recommend value of 0.4 by European legislations (Domínguez *et al*., 2016) the CR and CA still exhibited superior values.

The atherogenicity (AI) and thrombogenicity (TI) indices in human intake can linked to an increase in cardiovascular and other chronic non-transmissible diseases, when these values are not as low as possible (Santos *et al*., 2021; Ulbricht and Southgate, 1991). The AI and TI of the *T. catappa* kernel oils from purple (CR) and yellow (CA) varieties were higher than the values from *Caryocar villosum* and *Oenocarpus bacaba* oils determined in the works of Lorenzo *et al*. (2020) and Pinto *et al*. (2018), and lower than *Bactris gasipaes* oil (Santos *et al*., 2020). The replacement of animal fats in reformulated meat products by vegetable oils with lower AI and TI have demonstrated a significant improvement from a nutritional perspective (Domínguez *et al*., 2016). The h/H ratio of CR and CA oils were similar, and lower than the other tropical fruit oilseeds. A high value for this index in lipid intake may be advantageous to reducing low-density lipoproteins (LDL) in cholesterol fractions (Santos-Silva *et al*., 2002).

The calculated oxidation capacity value, COX, has a strong correlation with the proportion of PUFAs in lipid sources, and therefore, is expected to be higher in oils with high contents of PUFAs because they are more susceptible to oxidation. The COX value was lower in the CR oil than CA oil, which could be explained by the significant difference in the amount of linoleic acid between them, higher in CA than CR. The COX values for both samples, CR and CA, were lower than the indices of 6.6, 7.3, 6.5 and 7.8, as determined in oils from non-conventional sources, black cumin seeds (*Nigella sativa*), grape seeds (*Vitis vinifera*), tomato seeds (*Lycopersicon esculentum*) and wheat germ (*Triticum vulgare*), respectively (Hassanien *et al.,* 2014). The CA and CR oils displayed lower COX values when compared to conventional oilseeds, such as linseed (12.6 - 13.9), sunflower (1.94 - 9.16), rapeseed (4.2 - 4.4), and camelina oils (8.7 - 9.4) (Symoniuk *et al.*, 2018).

Table 3. Nutritional quality indices of *Terminalia catappa* kernel oils from purple (CR) and yellow (CA) varieties and other tropical fruit oilseeds

| ***T. catappa* oils** | | **Other tropical fruit seed oils** | | |
|---|---|---|---|---|
| **CR** | **CA** | ***Caryocar villosum*[1]** | ***Bactris gasipaes*[2]** | ***Oenocarpus bacaba*[3]** |
| 0.63 | 0.68 | 0.61 | ND | 0.43 |
| 0.50 | 0.54 | 0.38 | 1.10 | 0.30 |
| 1.16 | 1.27 | 0.75 | 2.04 | 0.67 |
| 0.75 | 0.79 | 2.58 | 0.84 | 3.32 |
| 2.75 | 3.05 | ND | ND | ND |

Data represent the calculated results from mean values (n = 3) of FA fractions, according to the equations previously presented. P/S – Polyunsaturated/saturated fatty acid ratio, AI – Atherogenicity index, TI – Thrombogenicity index. *h/H* – Hypocholesterolemic/hypercholesterolemic ratio, COX - calculated oxidation value. ND – non-determined. [1]Lorenzo *et al.* (2020); [2]Santos *et al.* (2020); [3]Pinto *et al.* (2018).

Furthermore, these data corroborate the advantageous use of the *T. catappa* kernel oils from purple (CR) and yellow (CA) varieties for agro-industrial applications.

### *3.2.3. Triacylglycerol composition*

The composition of triacylglycerides (TAGs) in CR and CA oils is displayed in Table 4. Both oils exhibited a quite similar proportion of triacylglycerols. The most frequently estimated triacylglycerols in CR were PLO, POO, POP, OLO, PLP, OLL and OOO, which represent 74.73% of the total. On the other hand, the predominant triacylglycerols in CA oil were almost the same, but slightly different with PLO, POO, POP, OLO, PLP, OLL and PLL representing 74.74% of the total. It was observed that the composition of TAGs was mainly composed of unsaturated acylglycerols, $SU_2$ and $U_3$, as can be seen in Table 4, which should be expected considering that the oleic and linoleic acids were most frequently in its FAs profile. Triglycerides are an important group of lipid sources for human nutrition. The TAG composition of CR and CA oils presented the predominance in ECN52 followed by ECN54, which can be linked to a large amount of long-chain triglycerides and, therefore, their inclusion in human intake can be helpful for preventing cardiovascular diseases.

Table 4. Estimated percentage of triacylglycerol composition of *Terminalia catappa* kernel oil from purple (CR) and yellow (CA) varieties.

| ECN | Triacylglycerol | *Terminalia catappa* oil % (normalized) | |
|---|---|---|---|
| | | CR | CA |
| C48:0 | PPP | 3.13 | 3.87 |
| C50:0 | SPP | 1.75 | 1.98 |
| C50:1 | POP | 11.80 | 11.77 |
| C50:2 | PLP | 6.94 | 9.06 |
| C52:1 | SOP | 4.41 | 4.02 |
| C52:2 | SLP | 2.59 | 3.09 |
| C52:2 | POO | 14.85 | 11.95 |
| C52:3 | PLO | 17.47 | 18.39 |
| C52:4 | PLL | 5.14 | 7.08 |
| C54:2 | SOO | 2.77 | 2.04 |
| C54:3 | SLO | 3.26 | 1.14 |
| C54:3 | OOO | 6.22 | 4.04 |
| C54:4 | SLL | 0.96 | 1.21 |
| C54:4 | OLO | 10.98 | 9.34 |
| C54:5 | OLL | 6.46 | 7.19 |
| C54:6 | LLL | 1.27 | 1.84 |
| **Triacylglycerol classes** | | **%** | |
| $S_3$ | | 4.88 | 5.85 |
| $S_2U$ | | 25.74 | 27.94 |
| $SU_2$ | | 44.45 | 41.81 |
| $U_3$ | | 24.93 | 22.41 |

ECN: equivalent carbon number. P - Palmitic acid, S - Stearic acid, O - Oleic acid, L - Linoleic acid.
S = saturated acylglycerol and U = unsaturated acylglycerol. Data represent the calculated results from mean values (n = 3) of FA fractions, according to the software PrOleos®.

### *3.2.4. Analyses of bioactive compounds and antioxidant capacity*

The bioactive compound analyses for anthocyanin, ascorbic acid and total polyphenol contents, and the antioxidant activity (TEAC assay), in the *Terminalia catappa* L. kernel oils from purple and yellow varieties are presented in Table 5. The analyses were used to investigate the presence of these bioactive compounds, and their potential antioxidant action as preserving agents in CR and CA oils. It was observed that the CR oil displayed higher values for bioactive compounds and antioxidant activity than CA oils ($p < 0.05$), which can be related to the difference in its variety.

In another work, the antioxidant activity of oils from *T. cattapa* was evaluated by the DPPH assay and its EC50 was found to be close to 7 $mg \cdot mL^{-1}$, indicating a potential antioxidant action (Ladele *et al*., 2016). Castelo-Branco, Torres (2012) investigated the antioxidant activity by TEAC assay of conventional oilseeds, such as soyabean, maize, sunflower, and canola, and found values close to 7.1, ~4.5, ~4.3 and ~5.3 mmol of Trolox eq$\cdot Kg^{-1}$ of oil, respectively.

A precise comparison of data from bioactive compounds and antioxidant activity in *T. catappa* oil was

Table 5. Bioactive substances and antioxidant capacity of *Terminalia catappa* kernel oils from purple (CR) and yellow (CA) varieties.

| Assays | Samples | |
|---|---|---|
| | CR | CA |
| Anthocyanin content ($mg \cdot 100\ g^{-1}$) | $18.3 \pm 1.5^a$ | $2.55 \pm 1.03^b$ |
| Ascorbic acid content ($mg \cdot 100\ g^{-1}$) | $68.48 \pm 2.02^a$ | $38.7 \pm 1.5^b$ |
| Total polyphenols content ($mg \cdot GAE\ g^{-1}$) | $152.3 \pm 2.4^a$ | $127.3 \pm 3.0^b$ |
| Antioxidant activity ($\mu g \cdot TE\ g^{-1}$) | $38.6 \pm 2.2^a$ | $31.1 \pm 1.6^b$ |

Data represent the mean ± standard deviation of triplicate analyses (n = 3). Different superscript lowercase letters in the same line represent significant differences between samples ($p < 0.05$) at 95% confidence interval according to Tukey's test.

difficulted because of the scarce information available. Furthermore, different results found in the literature can be related to differences in protocol, sample preparation, solvents used, variations among species, harvest season, environmental conditions, and others.

## 4. CONCLUSIONS

The solvent extraction of *Terminalia catappa* L. kernel oils from purple (CR) and yellow (CA) varieties showed a good yield, above 54% lipids. These unconventional oils presented high-quality physical-chemical parameters, mainly observed by low levels of acidity and peroxides. Both oils, purple and yellow varieties, exhibited the predominance of unsaturated fatty acids (UFAs), with almost 63% oleic and 26% linoleic acids, which influenced its nutritional quality index values. These oils presented higher values for polyunsaturated and saturated acids ratio, which is relevant to human diets. The atherogenicity and thrombogenicity indices were higher in the *T. catappa* oils when compared to other tropical oilseeds. The calculated oxidation capacity values for both oils were lower than other non-conventional oil sources, even with a high proportion of PUFAs. The composition of triacylglycerols was mainly composed of unsaturated acylglycerols, which may be helpful for preventing cardiovascular diseases with their inclusion in human intake or in a potential use in formulated products with improvements in their nutritional profiles. Besides the nutritional quality properties, *T. catappa* oils from both varieties exhibited significative contents in anthocyanin, ascorbic acid and total polyphenol contents, and good antioxidant activity as determined by the TEAC assay. Thus, the presented results confirm the importance of *T. catappa* as a lipid source for human intake and to be used in the development of food products.

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