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Chemical Characterization and Stability of the *Bombacopsis glabra* Nut Oil

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Abstract The aim of this study was to characterize the *Bombacopis glabra* nut oil (Malvaceae-Bombacoideae) by the determination of its lipid content and fatty acid composition with emphasis on the cyclopropenoid fatty acids (CPFA). The lipids were obtained by five different extraction conditions:[raw almonds: maceration with ethyl ether (I-MA) and n-hexane (II-MA), both at room temperature, and Soxhlet extraction with hexane for 6 (III-MA) and 12 h (IV-MA) and toasted almond: maceration with hexane at room temperature (V-MA)]. Additionally, the oxidation stability of oil by the Rancimat test and the boiling point by thermal analysis (technical TG / DTG) were evaluated. The oil content ranged from 34.99 (I-MA) to 47.36% (IV-MA); oxidation stability was 4.18 h and the boiling point was 373.37 ℃. It should be noted that results about thermal and oxidative stability are been reported for the first time with respect to *Bombacopis glabra* nut oil. The major oil constituents were palmitic acid (56.06%) and estercúlico (24.83%). The high percentage of CPFA oil, determined by NMR ^{1}H (26.2 to 30.9%) and GC-FID (26.5%), reinforce that the kernels of this species are not suitable for human consumption.

Keywords Cyclopropenoid Fatty Acids, Oxidation Stability, Thermogravimetric Analysis

1. Introduction

Bombacopsis glabra (Pasq.) A. Robyns belongs to the Malvaceae – Bombacoideae family, it presents as synonyms *Pachira glabra* Pasq., *Pachira macrocarpa* (Schlecht. et Charm.) Walp, *Bombax glabrum* (Pasq.) A. Robyns and *Bombax aquaticum* (Aubl.) Schum.[1,2]. This plant is popularly known in Portuguese as "castanha-do-maranhão", "mamorana", "castanha-da-praia", "cacau-do-maranhão" and "cacau-selvagem". It occurs in tropical and subtropical regions of the America and Europe[3], being native from the Atlantic pluvial forest, from the States of Pernambuco to Rio de Janeiro, as well as in the Amazon, where it grows on the river banks, streams and Amazon River estuary.

B. glabra is an arboreal ornamental plant, measuring from 4 to 6 m high. The seeds are the key propagation means, with 100% of germination, which occurs from five to ten days after its seeding. The flowering occurs from September to November, with the fruit maturation at the beginning of the year[3,4]. This species can be used in the recovery of degraded areas, with a good development of the seedlings under full sun, tolerating 30 to 50% shading, moreover, it is used as living stake ("mourão"), by the assorian communities of Santa Catarina Island, Brazil.

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The fruit contains an average of 18 seeds rich in pleasant flavored oil, consumed by man and wild animals; however, it is still little studied as regards its economical utilization[5]. The annual production is approximately 63 fruit per plant, which corresponds to an estimated 570 kg of seeds per hectare, with a total of 400 individuals[6].

Species of the Malvaceae family have as a common characteristic the presence of triacylglycerols of cyclopropenoid fatty acids (CPFA) in their seeds and nuts oil[2]. Cyclopropene ring-containing compounds are associated to several biological effects on animals, including carcinogen and co-carcinogen activities[7-9].

The most common cyclopropenoid fatty acids (CPFA) are the malvalic (7-(2-octacyclopropen-1-yl)heptanoic acid) and sterculic (8-(2-octacyclopropen-1-yl)octanoic acid). This last one inhibits the enzyme Δ^9-desaturase, which converts the stearic acid into oleic acid, and it is potentially noxious to humans also being able to alter the permeability of the membranes and inhibit the cell reproduction[10].

Breyne[6] observed that in some studies on the chemical composition of the *B. Glabra* oil, the percentage of cyclopropenoid fatty acids (CPFA) ranged from 24.5-34% and, in others there was no mention about the existence of these substances. According to reports in the literature, the divergence in the results of the analysis may be due to the instability of the CPFA, which can be decomposed by heat and acid, and therefore depends on the manner of extraction, the derivatization conditions and the chromatographic analysis[8,9].

Regarding the discrepancies on the content of CPFA in *B. glabra* nuts, the possible undesirable effects related to the consumption of these fruit and the possibility of the Brazilian existing species consist of a genotypically distinct population of plants studied previously, the current work aimed to characterize the *Bombacopis glabra* nut oil (Malvaceae-Bombacoideae) cultivated in the region of Brasília-DF, capital city of Brazil, by the determination of its lipid content extracted under different conditions. In addition, fatty acid composition with emphasis on the ciclopropenoídicos (AGCP) was determined, as well as evaluated for the first time thermal and oxidation stability of the oil.

2. Materials and Methods

2.1. Instrumentation

The methyl esters were analyzed on a gas chromatograph with a flame ionization detector (GC-FID), model GC17A, Shimadzu equipped with a SELECT FAME column, Varian, and on a gas chromatograph model GC17A, with a mass detector (GC-MS), model QP5000, Shimadzu, column: SP 2560, SUPELCO. The nuclear magnetic resonance (NMR) analyses were obtained in a Bruker Avance DRX-500 spectrometer and Varian Inova 500, and the thermogravimetric analysis on a TGA-2050 thermogravimetric balance, from TA Instruments. The determination of the stability to oxidation was carried out on the Rancimat Metrohm 743.

2.2. Vegetal Material

Bombacopsis glabra (Pasq.) Robyns nuts were collected in June 2006, in Brasília-DF, and stored in paper bags at room temperature. The botanic identification was accomplished by Dr. Carolyn Elinore Barnes, from Brasília University-UnB, Brasília, Brazil. A voucher specimen has been deposited in the UnB Herbarium under reference UB 76691.

2.3. Oil Extraction

B. glabra nut were ground and oil extracted, according to the following procedures: Two samples, of approximately 10 g of nuts, were extracted by maceration. One extract was obtained with 50 mL of ethyl ether (I-MA) and the other one (II-MA) with 50 mL of hexane, for 24 hours at room temperature. Two samples, of nearly 10 g of nuts, were extracted with hexane, on Soxhlet. One sample was extract for 6 hours[11] and the other one, for 12 hours**,** yielding the extracts III-MA and IV-MA, respectively. The seeds were toasted in a microwave oven at maximum power for two minutes. After the shell removal, 10 g of nuts were ground and submitted to extraction with 50 mL of hexane at room temperature, yielding extract V-MA. The extracts were filtered; the solvent removed in a rotary evaporator at 50℃, and the oil kept in a desiccator until its constant weight.

2.4. Determination of CPFA by ^{1}H NMR

The ^{1}H NMR spectra was obtained from 50 mg of the oil *in natura* dissolved into 0.6 mL of $CDCl_3$, using tetramethylsilane (TMS) as an internal reference standard, at the frequency of 500 MHz. The percentage of cyclopropenoid fatty acids was determined using the expression:

$$\%CPFA = (100x3A_{CH_2}) / 2A_{CH_3} \quad (1)$$

in which A_{CH2} represents the integration of the singlet area in δ 0.77, referring to the methylene hydrogens of the cyclopropene ring of the CPFA and A_{CH3} represents the integration of the triplet area in δ 0.89, corresponding to the methyl hydrogens of all fatty acids composing the triacylglycerols of the oil[8,9].

2.5. Determination of Fatty Acid Methyl Ester, Including CPFA, by CG/FID and GC/MS

The oil extracted with ethyl ether at room temperature (extract I-MA) was transesterified according to the methodology previously described[9,12]. The fatty acid composition was determined by GC-FID and the CPFA confirmed by GC-MS. The compounds were separated on a 100 m capillary column. The analysis conditions by GC-FID were as follows: initial temperature of the column: 45℃ (4 min); heating rate: from 13℃ min^{-1} to 175℃ (27 min) and from 4℃ min^{-1} to 215℃ (9 min). Injector and detector temperature: 250℃, carrier gas: hydrogen, flow: 1.9 mL min^{-1}, linear velocity: 34 cm s^{-1}, pressure at the column top: 175 kPa. The quantification was carried out by area normalization and the results expressed in % m/m of methyl esters. The GC-MS analysis were carried out on the following conditions: scan mode; initial temperature of the column: 190℃ (60 min); heating rate: from 10℃ min^{-1} to 220℃ (30 min), injector and interface temperatures: 225℃ and 230℃, respectively; carrier gas: helium, flow: 0.7 mL min^{-1}, linear velocity: 17.7 cm s^{-1}, split ratio: 1:50 and pressure at the column top: 216 kPa. The mass fragments were obtained by electron impact, with energy of 70 eV, and the analyzer was a quadrupole type. The identification of the components was carried out by comparison with the spectra from the GC-MS library (Nist 62 and Nist 12) and by comparison with the published mass spectra of the methyl esters of the malvalic and sterculic acids 9].

2.6. Thermogravimetric Analysis of the Oil

The TG/DTG curves of the extracted oil with hexane at room temperature were obtained within a temperature interval of 30-600℃, with a heat ratio of 10℃ min^{-1}, in a nitrogen atmosphere with an output of 50 mL min^{-1}, using an aluminum pot of 20 μL, with an approximately 0.5 mm hole in the lid. The mass loss of the sample was established as the difference between the initial and the final mass. The boiling point ("onset" temperature) was considered as the intersection point of the tangent of the mass loss inclination with the initial base line. The software of the equipment was used to draw the tangent lines and record the boiling temperature[13,14].

2.7. Oxidation Stability Test

The stability to oxidation was measured on the Rancimat at 110°C and a constant airflow of 10 L h^{-1}. A sample of 3 g of oil extracted with hexane at room temperature was used. On the Rancimat, the airflow passes through the sample, being bubbled afterwards in a flask containing deionized or distilled water. The airflow carries the volatile carboxylic acids (oxidation outputs), which solubilize and increase water conductibility. The obtained response is an electrical conductibility curve versus time, in which two tangents are built that intercept each other in a point, corresponding, in the time scale, to the induction period or stability to oxidation[15].

2.8. Statistical Analysis

The analyses were carried out in triplicate, and the averages and standard deviation of the measures were calculated. The ANOVA test was applied, using the Microcal Origin 7.5 to evaluate the difference between the averages of the percentages of CPFA obtained for the samples.

3. Results and Discussion

3.1. Characterization of the Oil

The *B. glabra* nuts, after the extraction by means of five experimental procedures, provided the following extracts coded as: I-MA, II-MA, III-MA, IV-MA and V-MA, with oil amounts ranging from 34,99 ± 4.94 to 47.36 ± 0.28% (Table 1). The highest oil yields were obtained from the III-MA (46.28%) and IV-MA (47.36%) samples, which resulted from the extraction of the nuts by Soxhlet for 6 and 12 hours, respectively.

The ^{1}H NMR spectra of the oils, obtained by different extraction procedures, presented a singlet in δ 0.77, attributed to the methylenic hydrogens of the cyclopropenic ring[8] and characteristic signals of the structure of triacylglycerols[CH_2O: δ 4.15 (*dd*) and 4.30 (*dd*); CHO: δ 5.35 (*m*)][16].

Table 1. Oil and cyclopropenoid fatty acids (CPFA) content in the *B. glabra* nut oil

Sample code	Oil mass (g) ± SD	Oil yield (%) ± SD	% CPFA* ± SD
I-MA	3.57 ± 0.41	34.99 ± 4.94	30.88 ± 3.67
II-MA	3.81 ± 0.45	38.65 ± 3.32	28.82 ± 1.98
III-MA	2.29 ± 0.08	46.28 ± 0.19	28.29 ± 2.97
IV-MA	2.30 ± 0.06	47.36 ± 0.28	26.18 ± 0.49
V-MA	3.51 ± 0.53	35.41 ± 4.98	29.84 ± 1.29

I-MA: oil extracted with ethyl ether at room temperature; II-MA: oil extracted with hexane at room temperature; III-MA: oil extracted on Soxhlet for 6 h; IV-MA: oil extracted on Soxhlet for 12 h; V-MA: oil extracted with hexane at room temperature, from roasted nuts in a microwave oven; CPFA: cyclopropenoid fatty acids. *Determined by ^{1}H NMR. SD: standard deviation.

The percentage of CPFA in the *B. glabra* oil, determined by ^{1}H NMR, ranged from 26.18 to 30.88% (Table 1), being consistent with those reported in the literature (24-34%)[6]. However, applying the ANOVA test, at a significance level of 5%, it was observed that there is no significant difference between the averages of the percentages of CPFA of all samples, independently on the extraction method.

Figure 1 presents the fatty acid profile of the *B. glabra* nuts crude oil, extracted with ethyl ether at room temperature and determined by GC-FID (A) and GC-MS (B). The malvalic acid (component 6) and the sterculic acid (component 10) were identified by GC-FID and by comparison with the profile obtained from the *Sterculia striata* oil, which contains cyclopropenoid fatty acids in their composition[8,9]; the confirmation was accomplished by GC-MS, through the comparison of the spectra obtained with the ones available at the Nist 62 and Nist 12 library, as well as with the mass spectra published for the methyl esters of the sterculic and malvalic acids[9].

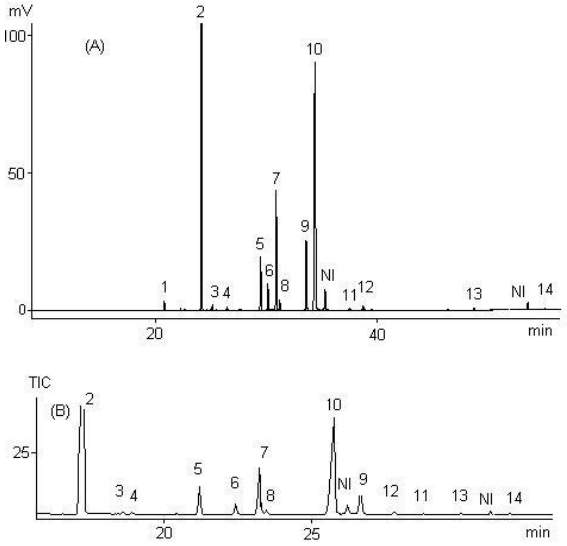

Figure 1. Chromatograms of methyl esters of fatty acids of *B. glabra* (castanha-do-maranhão) nut oil obtained in a gas chromatograph: (**A**) flame ionization detector; (**B**) mass detector. 1. Myristic acid (14:0); 2. Palmitic acid (16:0); 3. Palmitoleic acid (16:1, 9*Z*); 4. Margaric acid (17:0); 5. Stearic acid (18:0); 6. Malvalic acid; 7. Oleic acid (18:1, 9*Z*); 8. Vaccenic acid (18:1, 11*Z*); 9. Linoleic acid (18:2, 9*Z*,12*Z*); 10. Sterculic acid; 11. Linolenic acid (18:3, 9*Z*,12*Z*,15*Z*); 12. Arachidic acid (20:0); 13. Behenic acid (22:0); 14. Lignoceric acid (24:0); NI: unidentified

Table 2 presents the *B. glabra* nuts crude oil fatty acid composition, determined by GC-FID. The levels found for most fatty acids were within the ranges described in the literature for the *B. glabra* oil, except for the linoleic and linolenic fatty acids. As regards the malvalic and sterculic acids, which contain cyclopropenic groups in the molecule, the obtained contents also corresponded to those described[6].

Comparing the results of the cyclopropenoid fatty acids contents determined by GC-FID (Table 2) and by ^{1}H NMR (Table 1), there was a similarity of values. By GC-FID the average sum of CPFA (malvalic and sterculic) was 26.5%, while by NMR, depending on the on the method of oil extraction, the levels ranged from 26.2 to 30.9%. It is noteworthy that the ^{1}H NMR analysis determines the sum of all

CPFA as by GC-FID were only quantified the CPFA identified, ie the malvalic and the sterculic. Moreover, it is reported in the literature the presence of other cyclopropenoid fatty acids in *B. glabra* seeds, such as the 2-hydroxy- sterculic acid[6].

The high percentage of CPFA in oil of *B. glabra* reinforce that the kernels of this species are not suitable for human consumption, however, it may be used for production of biofuels (biodiesel and bio-oil), as reported for the oil of *Sterculia striata*, which presents in its composition CPFA[17].

Table 2. Composition of fatty acids determined as methyl esters of the *B. glabra* nut oil obtained by GC-FID (%m/m of methyl esters)

Fatty acid	% ± SD
Myristic, 14:0	0.23 ± 0.01
Palmitic, 16:0	53.06 ± 0.60
Palmitoleic, 16:1 (9*Z*)	0.23 ± 0.03
Margaric, 17:0	0.16 ± 0.00
Stearic, 18:0	3.45 ± 0.05
Malvalic*	1.66 ± 0.08
Oleic, 18:1 (9*Z*)	7.42 ± 0.06
Vaccenic, 18:1 (11*Z*)	0.66 ± 0.00
Linoleic, 18:2 (9*Z*,12*Z*)	4.73 ± 0.06
Sterculic*	24.83 ± 0.50
Linolenic, 18:3 (9*Z*,12*Z*,15*Z*)	0.20 ± 0.00
Arachidic 20:0	0.38 ± 0.01
Behenic, 22:0	0.09 ± 0.00
Lignoceric C24:0	0.07 ± 0.00
NI	2.33 ± 0.19
Total cyclopropenoid fatty acids (TCPFA)	26.49
Total saturated fatty acids (TSFA)	57.44
Total unsaturated fatty acids (TUSFA)	13.24
*Identified by GC-MS (Nist 62 and Nist 12) and by comparison with the mass spectra published for malvalic and sterculic methyl esters fatty acid[9]; NI: Unidentified; SD: standard deviation.	

3.2. Thermogravimetric Analysis

The thermogravimetric analysis has shown as a fast technique to measure the boiling point and the vapor pressure of many organic compounds, including vegetal oils or alkyl esters of vegetal oils. According to Goodrum[13], this method does not show any visible evidence that the samples of methyl esters and triacylglycerols of fatty acids decompose before or after boiling.

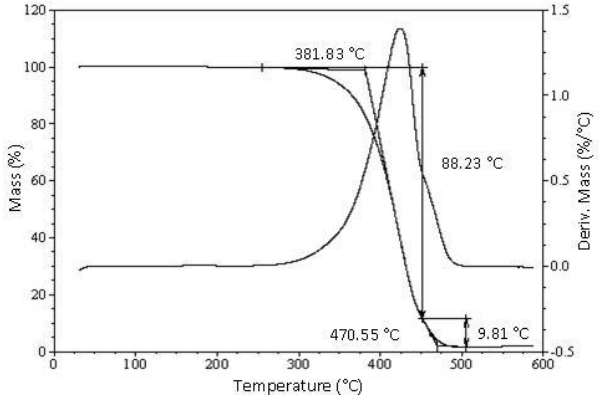

Figure 2. TG/DTG curve of the *B. glabra* oil extracted with hexane at room temperature

On the TG/DTG curve of the crude oil of *B. glabra*, extracted with hexane at room temperature (Figure 2), a mass loss of 88.23% was detected within the temperature interval 255.73-451.74℃, with an "onset" temperature (T_{onset}) of 381.83℃, referring to the boiling point of the triacylglycerols. A second mass loss of 9.81% occurred between 451.74-505.47℃, attributed to the volatilization of triacylglycerols of the largest chain and/or minor constituents present in the oil. It is pointed out that the TG/DTG curve of the *B. glabra* oil was very similar to the one observed for other vegetal oils, such as the babassu oil[18], castor oil[19] and *Dipteryx lacunifera* oil[20,21], as well as the *Sterculia striata* oil[22], which contains around 15% of CPFA[8]. Although the CPFA content in the *B. glabra* oil is approximately twice the one observed for *S. striata*, in the conditions used for the thermogravimetric analysis, no alterations were evidenced that could be attributed to the degradation of such substances.

3.3. Oxidation Stability

The *B. glabra* oil presented an oxidation stability as measured by the Rancimat induction period of 4.18 h, close to the value reported by Ferrari and Souza[23] for sunflower oil (4.47 h). The oxidation stability of an oil is influenced by the polyunsaturated fatty acids, linoleic (18:2) and linolenic (18:3), components of acylglycerols, which derive from the presence in the hydrocarbon chain of *bis*-allylic methylene groups (CH_2 neighboring the double bonds), which are more reactive than the allylic in the reaction with radical formantion by atmospheric oxygen. The products formed in this reaction are organic peroxides that decompose giving rise to minor hydrocarbon chain oxidized compounds, which contribute to the reduction of oxidation stability[24,25].

Dewick[10] reports the sunflower oil with a chemical composition ranging from 50 to 70% of linoleic acid (18:2), justifying thus the oxidation stability described by Ferrari and Souza[23]. In the *B. glabra* oil, the percentage of polyunsaturated fatty acids (18:2 and 18:3) is only 4.93%, therefore, the stability to oxidation of 4.18 h observed suggests it results, chiefly, from the instability of the cyclopropenoid fatty acids (malvalic and sterculic; 26.49%) present in the chemical composition of this species oil.

4. Conclusions

Nuts of *B. glabra* presented a high content of lipids (34.99 to 47.36%), although they are not suitable for human consumption due to the presence of harmful substances, such as triacylglycerols of cyclopropenoid fatty acids.

The main fatty acids of the triacylglycerols of the *B. glabra* oil were the palmitic, followed by sterculic, oleic, linoleic and malvalic acids.

The boiling point of the oil was 373.37℃, determined by a technique TG/DTG, but under the conditions studied was not detected any event attributed to the degradation of the triacylglycerols of cyclopropenoid fatty acids. However, the

oxidation stability was only 4.18 h. It should be emphasized that results about thermal and oxidative stability are been reported for the first time for *Bombacopis glabra* nut oil.

The obtained results represent an important contribution in order to discourage the indiscriminate consumption of the *B. glabra* nuts, an evidenced fact through the free trade, through the Internet, of the seeds for nourishing purposes.

The results confirm that the specie studied is genotypically similar to those reported in other studies even if not reported the presence of cyclopropenoid fatty acids. These components have not probable been recorded due the conditions of analysis not appropriated.

Considering that the *B. glabra* nuts are not suitable for the human nutrition, it is suggested that they be used for other economic purposes, such as the biodiesel production, however, it is necessary a more accurate survey for this applicability.

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