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Physico-chemical characteristics and oil content variability of seeds of four baobab species

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Abstract
In this study, the genetic variety of baobab seed oil on physicochemical characteristics was evaluated. Four species were selected for their genetic diversity (*Adansonia za, grandidieri, rubrostipa and digitata*) and their availability in different regions of Madagascar. The results indicated that the extraction yield of baobab oil was significantly impacted by seed variety. Seeds constitute 25-42% of the fruits, have low moisture content (4-8%). Their oil content varies from 28 to 71%. For the different oils extracted, their relative density are from 0.848 to 0.856; refractive index from 1.454 to 1.465. The average values are between 45.03 and 63.75 for iodine index; 147.96 and 176.76 mg KOH/g oil 1.454 and 1.465 for saponification value, 0.24 and 0.81 mEq O_2/ kg of oil for peroxide and 0,56 and 8,36 mg KOH/g oil for acide value. Gross disparities between species were noted. The results suggest that baobab oils are interesting and respect the standards recommended by the food code.

Keywords: Baobab seed oil, *Adansonia za*, *Adansonia grandidieri*, *Adansonia rubrostipa*, *Adansonia digitata*

Introduction

Baobabs of the genus *Adansonia* are dominant ligneous plants, which are characteristic of "dry" tropical forest ecosystems in sub-Saharan African countries, in northwestern Australia, in the south and along the western coast of Madagascar [1, 2]. In many regions of Africa and Madagascar, the baobab is an emblematic tree with various vital functions for the populations. Indeed, it can be a shelter, as well as having medicinal virtues but especially it has an essential nutritional function [3–5]. The baobab produces fruits whose pulp and seeds are consumed by the population [6–8]. The fruits are large, indehiscent berries of fairly variable shape and size. The kidney-shaped black to dark brown seeds are embedded in a dry, chalky, white to cream pulp, and are variable in size and weight depending on the species [9]. Although the fruits are widely exploited for their pulp, the use of the seeds remains marginal, the valorization of the latter is still too under exploited [4, 10, 11]. However, the seeds provide an oil that can be used in food or pharmaceutical. Indeed, these oils are known to be used in cosmetics, in pharmacology notably to treat skin diseases and inflammations of the gums and teeth, as well as in food [12, 13]. The population living near the baobab ecosystem extracts the oil from the seeds and uses it as food fat. Its rich and varied composition in fatty acids makes it a very interesting oil [11]. Baobab seed oil is non-siccative with an iodine value of 87.9 g/100 g, it contains phytosterols and in particular β-sitosterol (80%) known to reduce DNA damage, free radical levels and increases a number of antioxidant enzymes [14, 15]. The most important fatty acids are palmitic (C16:0), stearic (C18:0), oleic (OA, C18:1), linoleic (LA, C18:2) and α-linolenic (ALA, C18:3) acids. However, OA, LA and palmitic acids are the most abundant [11]. However, the rest of its composition remains to be elucidated.

In a context of valorization, the present study focused on baobab seed oils. Four species were selected, three endemics to Madagascar (*Adansonia grandidieri, rubrostipa, za*) and the African species *digitata*. The main objective is to determine the existing difference between the species and to target the most interesting species at the nutritional level. The specific objectives are to estimate the oil yield of the seeds and then to study the physicochemical parameters of these fats.

Materials and Methods

1. Collection of fruits

Baobab fruits were collected from four species selected for their genetic diversity (*Adansonia za, grandidieri, rubrostipa* and *digitata*) and their availability in different regions of Madagascar. The harvest areas and number of trees retained after sorting are presented in Table 1. Fruits of the same species were collected from different plants. At each collection site, trees of the same species at least 1 meter in diameter were identified. From each stand, 20 apparently healthy and mature pods were collected whenever possible. Upon collection in the field, the fruits obtained from each stand were stored in an individual labeled bag. This method was valid for all collections for each species and in each site. In the laboratory, the pods were spread out in a solar dryer for 24 hours to eliminate/reduce possible traces of water on the walls of the fruits, then repackaged in their respective transport bags until the time of use. Everything is placed in a dry place.

Table 1: Collection sites and periods

Species	Region	Area	Harvest period	Number of trunk	Geographical indications
grandidieri	Sud-ouest	Befandriana-sud	October 2018	9	22°6'9.2''S -43°53'36.8''E
	Menabe	Andranomena	November 2018	10	20°10'27.1''S - 44°30'9.1''E
	Menabe	Ampataka	November 2018	7	20°7'26.8''S - 44°26'51.7''E
rubrostipa	Menabe	Mangily	September 2019	9	20°52'22.9''S - 44°24'14.2''E
za	Diana	Ambanja	June 2019	2	13°52'43.7''S - 48°32'02.9''E
	Menabe	Anivorano	October 2019	6	20°11'21.0''S - 44°26'12.7''E
	Sud-ouest	Ampanihy	June 2019	10	24°41'26.4''S - 44°44'22.3''E
digitata	Boeny	Mangatsa	July 2019	9	15°37'30.6''S - 46°25'10.1''E

2. Estimation of the proportion of seeds

Each whole fruit was weighed, peeled and de-seeded to obtain the pulp and seeds separately (Figure. 1). The seeds were then weighed in turn.

Mature fruit

Seeds

Fig 1: Fruit and seed of *Adansonia grandidieri*

3. Determination of seed moisture and oil extraction

Seeds were dehulled and the resulting kernels ground. The moisture content of this powder (H%) was determined by loss of weight on drying a given quantity of a test sample, placed in an oven at 103°C±2 until a constant weight was obtained *[23]*. The oil was extracted with hexane by Soxhlet extraction using NF V03-908 method *[24]*. The hexane was removed using a rotary evaporator under vacuum. The oil yield is calculated on the ratio of oil mass obtained/kernel mass. The oil was collected in a stained-glass bottle, labeled and placed in a refrigerator (4°C) before using.

4. Determination of the physico-chemical parameters of oils

4.1. Relative density D

The relative density at 20°C of an oil is the density which is the ratio of the mass of a certain volume of oil at 20°C, to the mass of an equal volume of distilled water at 20°C. The determination was made with a pycnometer according to ISO 6883: 2017 [25].

4.2. Refractive index I_R

Refractive index I_R is the ratio between the sine of the angles of incidence and refraction of a light ray of a given wavelength, passing from the air into the oil maintained at a constant temperature. The determination is made according to ISO 6320: 2017 [26]. The measurement was made with a ABBE refractometer.

4.3. Iodine Index I_I

The iodine value (I_I) provides information on the degree of unsaturation of the fatty acids in a given oil. The iodine value is defined as the mass of iodine absorbed by the oil. It has been determined according to the ISO 3961:2018 [27].

4.4. Acid index I_A

The acid number (I_A) is a tool to assess the quality and monitor the state of deterioration of fats. When fats become rancid, triglycerides are converted into fatty acids and glycerol, resulting in an increase in the I_A. The measurement was done according to ISO 660: 2020 by backflush [28].

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4.5. Saponification index

The saponification index I_S is the number of milligrams of potassium hydroxide required to saponify one gram of oil. This parameter was determined according to the protocol described by the ISO 3657: 2020 [29].

4.6. Peroxide value

The double bonds in the oil are fragile areas to the oxidation of air, giving rise to peroxides. The formation of peroxides is accompanied by the appearance of secondary products (aldehydes, ketones, acids and peroxides) responsible for rancidity. The peroxide index I_P of a lipid allows to appreciate its conservation qualities. It is the number of milliequivalents of active oxygen contained in a kilogram of oil and likely to oxidize potassium iodide. It has been determined according to the ISO 3960: 2017 [30]*).* Higher the index is, more the fat is oxidized.

5. Statistical analysis

Data were expressed as means ± SD. All physicochemical assays were performed in triplicate independent experiments. Statistical analysis was achieved using XLSTAT software. Significant differences ($p < 0.05$) between the means were determined by analysis of variance (ANOVA). The comparison was made between species.

Results and Discussions

Proportion of seeds in *Adansonia* fruits

Fruit weights vary significantly by species. Considering the average weight (Table 2), *digitata* fruits are the heaviest (356.54 g), while *rubrostipa* has lighter fruits (106.16 g). The seeds' weight is proportional on fruit's weight. When

expressed as percentages, seeds constitute between 26 and 42% of the total mass of the fruit; the *grandidieri* species has the highest proportion (42.47%) followed by *rubrostipa* (40.68%), *za* (36.82%) and finally *digitata* with 25.96%. The proportion of seeds is variable according to the species. Particularly for *digitata*, this value falls within the ranges found in other work [33]. Indeed, seed/fruit ratios ranging from 23 to 34% have been observed for the same species, and those associated with significant morphological and biochemical diversity depending on the agroclimatic zones of origin of the fruit in Cote d'Ivoire.

Table 2: Mass of *Adansonia* fruits and seeds by species

Species	Number of observations	Mass of fruit (g)			Mass of seeds (g)			Mass percentage of seeds (%)		
		Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
grandidieri	130	37.90	534.90	159.24 ± 77.02 ^{c}	10.90	285.60	70.69 ± 41.89^{c}	28.75	53.40	42.47 ± 7.94^{a}
rubrostipa	80	42.60	175.30	106.16 ± 37.30 ^{d}	19.60	90.10	44.26 ± 20.36^{d}	13.85	60.46	40.68 ± 10.90^{b}
za	80	120.00	437.00	197.93 ± 64.71 ^{b}	23.40	147.80	73.22 ± 28.09^{b}	9.48	49.37	36.82 ± 7.33^{c}
digitata	80	150.60	602.10	356.54 ± 24.88 ^{a}	8.20	213.20	97.38 ± 51.27^{a}	5.44	41.89	25.96 ± 7.68^{d}

Mean ± standard deviation of results

Values in the same column followed by different letters are significantly different (p value <0.05)

3. Water content, dry matter and oil yield of seeds

Seed moisture content (**Table 3**) for all species is low, ranging from 4% for *grandidieri* to ~8% for other species. Therefore, this low moisture content reflects a high dry matter content of 92-96%. This low level of moisture associated with adequate storage conditions has the advantage of slowing down any microbial development, limiting reactions that could cause a rapid degradation of seed components, especially lipids, and therefore ensuring a relatively long storage time. Some authors *[36]* have found similar values by working on the same species which grew in broadly different ecosystems. Their water content at harvest time were : *grandidieri 4.7% rubrostipa* 4.7 – *za* 7.7 digitata 6.1.

The oil yield varies considerably according to the species; *grandidieri* shows the highest value 71%, 40% for *rubrostipa*, 37% for *za* and. 28% for *digitata*. According to Table 2, as the mass percentage of seeds increases, the oil yield increases. Previous work on *grandidieri* and *rubrostipa* species using the same extraction method reported 43.3% - 44.4% and 19.4% - 21.1% respectively (16,17). The yield is significantly lower if the extraction is carried out in an artisanal way unless heat treatment is applied (45.2% for *grandidieri*) [16].

In addition to the degree of maturity of the seeds and the climatic and edaphic conditions, the oil yield is thus a function of the extraction method. Heat treatment/roasting of the seeds could be considered to improve these rates because it favors the release of lipids and to deactivate beforehand the enzymes contained in the seeds. However, its application would have to be monitored because it could lead to a possible alteration of the quality of the oil leading to the formation of peroxides and browning reactions.

These contents observed here largely exceed those of oleaginous seeds such as soybean (17-21%), sunflower (25-40%), moringa (41%) *[37]*. Seeds are thus a source of lipids and could constitute a potential raw material for oil milling, provided that the cycloprenic fatty acids present are properly eliminated.

Table 3: Water, dry matter and oil content of seeds

Species	Water content (g/100g FM)	Dry matter (g/100g FM)	Oil yield (g/100g FM)
grandidieri	4.10 ± 1.01^{b}	95.81 ± 1.01^{b}	71.14 ± 6.49^{a}
rubrostipa	7.55 ± 0.46^{a}	92.44 ± 0.46^{a}	40.79 ± 0.62^{b}
za	8.60 ± 0.35^{a}	91.39 ± 0.35^{a}	37.10 ± 0.60^{bc}
digitata	8.91 ± 0.26^{a}	91.08 ± 0.26^{a}	28.46 ± 0.89^{c}

Mean ± standard deviation of results.

Values in the same column followed by different letters are significantly different at the 95% confidence level (ANOVA); (p value <0.05).

4. Physico-chemical properties of oils

Data on the physicochemical properties of the seed oil are presented in Table 4.

Table 4: Physico-chemical characteristics of *Adansonia* seed oils

Species	*grandidieri*	*rubrostipa*	*za*	*digitata*	Codex standard [1]
Density at 20°C	0.856± 0.006^{a}	0.848± 0.06^{a}	0.857 ± 0.01^{a}	0.855± 0.07^{a}	
Refractive index at 20°C	1.455± 0.004^{b}	1.452± 0.00^{b}	1.465 ± 0.00 ^{a}	1.454± 0.00^{b}	
Iodine Index	45.03± 3.94^{c}	56.22± 0.05^{b}	59.86 ± 0.3^{ab}	63.75± 0.06^{a}	
Acid value (mg KOH/g oil)	0.56 ± 0.07^{d}	4.06 ± 0.02^{b}	8.36 ± 0.03^{a}	2.12 ± 0.01^{c}	4 [1]
Saponification value (mg KOH/g oil)	147.96± 15.07^{b}	165.27 ± 0.00^{ab}	176.76 ± 0.00^{a}	158.27 ± 0.00^{ab}	
Peroxide value (mEq O2/ Kg of oil)	0.81± 0.05^{a}	0.26±0.01^{c}	0.35 ± 0.04^{b}	0.24± 0.02^{c}	10 [1]

Mean ± standard deviation of results.

Values in the same row followed by different letters are statistically different at the 95% confidence level; (p <0.05)

(1) CXS 19-1981 [31]

4.1. Density of oils

The density of the oils varied from 0.848 to 0.857. Statistically, the differences are not significant between the species. There is no standard for this oil yet, making comparison and interpretation difficult, but if we refer to the density of soybean oil (0.919-0.925), which is known to be rich in UFAs, that of baobab is lower. As the density of an oil increases with the average length of the fatty acid chains.

These values indicate that baobab oils are rich in low molecular weight fatty acids.

4.2. Refractive index

The I*R* varies according to the unsaturation of the oil, and is influenced by many other factors such as free acidity, oxidation, polymerization and the existence of secondary functions on the molecules. It is proportional to the molecular weight of the fatty acids as well as to their degrees of unsaturation. A high refractive index allows to conclude the presence of double bonds. The I_R value of baobab oils varies from 1.452 to 1.465; z*a* has an I_R close to that of soybean oil (1.466) and the other three species are more like palm or palm kernel oil as specified in CSX 210-1999 *[32]*. The composition in GA would be different according to the species considered.

4.3. Iodine Index I_I

I_I provides information on both the degree of unsaturation of the fatty acids contained in a given oil and on the dryness of the oil. It is related to the degree of oxidation of an oil. Thus, the more unsaturated an oil, the higher its I_I. This index could be used as a basis for assessing the ease of rancidity of the oil, since the more unsaturation it contains, the more sensitive it will be to oxygen.

The values obtained: 45.03 to 63.75g/100g of oil, allow us to classify baobab oils among the non-drying oils, whose II are lower than 110 *[17]*. The *grandidieri* species has the lowest value while *digitata* has the highest. These low values of iodine index could mean a low amount of unsaturated fatty acids in the oils and, in this case, a preservation that can be done without the risk of rapid auto-oxidation. Authors have reported II values ranging from 57 to 96 g/100g of oils on different baobab species *[18,19]*.

The values are far from those of common consumer oils (soybean: 124-139, sunflower: 118-141, peanut: 77-107, but tend to approach those of palm: 50-55) *[32]*.

4.4. Acid index I_A

The I_A reflects the level of free fatty acids in the oil as a result of triglyceride hydrolysis, one of the causes of oil spoilage. Food code CXS 19-1981 *[31]* limits the maximum I_A value for edible fats and oils not covered by individual standards to 4 mg KOH/g oil. The I_A values of *grandidieri* (0.56mg), de) and *digitata* (2.12mg) oils appear to be complying, giving them good stability. That of *rubrostipa* (4,06mg) is at the limit. On the other hand, this criterion is not satisfied for the oil of *za*, with 8,36mg. However, RALAIMANARIVO *et al.* reported an acid value of 2 to 2.8mg for this same species *[18]*. These disparities of values observed by species compared to those of other authors do not allow to conclude to a genetic effect but rather that these different I_A could be attributed to the delay of preparation of the samples and extraction of oils and storage before the analyses in series and which do not rule out the possibility of a beginning of lipolysis It is therefore necessary to proceed to a refining or a heat treatment before extraction to limit a rapid post-extraction denaturation.

4.5. Saponification index

The I_S provides information on the length of the carbon chain of fatty acids constituting the oil. It is higher the shorter the carbon chain of the fatty acids *[20]*. The SI of the oils obtained varies between 147.96 and 176.76mg KOH/g oil; *grandidieri* is the lowest and *za* the highest. For all the oils analyzed, the saponification index is low, suggesting that these oils contain long chain fatty acids.

These values are lower than those reported by RALAIMANARIVO *et al*. *[18]*, between190 and 195 mg KOH/g oil, and deserve to be reviewed, as they are in favor of a long-chain fatty acid composition, which contradicts the results reported by other authors for the same species where medium-chain fatty acids such as palmitic acid predominate *[21]*.

The saponification value of baobab oils is lower than those of peanut (187 to 196mg KOH/g oil), soybean (189 to 195mg KOH/g oil) and sunflower (188 to 194mg KOH/g oil) oils, but is rather close to those of rapeseed oil (168 to 181mg KOH/g oil) *[32]*.

4.6. Peroxide value

It is a useful criterion for assessing the early stages of oxidative deterioration of an oil *[22]*. The I_P values of baobab oils found in this study are 0.24 to 0.81mEq O_2/Kg of oil for *digitata* and *grandidieri* respectively. These values are well below 10 mEq O_2/Kg of oil, the quality factor that characterizes most conventional oils *[32]*. This value also confirms a low level of oxidation.

It should be noted that these oils have not undergone refining and therefore retain minor unsaponifiable fractions with antioxidant activities and confer quality protection, and maintenance of the composition of the oil or a lower risk of deterioration or disappearance of essential nutrients. In general, these minor constituents of the oil with anti-free radical effect are essential to prevent the oxidation of double bonds and delay peroxidation.

Conclusion

The physicochemical characteristics showed that the baobab oils are interesting and conform to the standards recommended by the food code. The values of the measured parameters seem to indicate that these oils are suitable for long conservation, under appropriate conditions. However, the absence of references for this type of oil or for a similar product of the same family makes comparison and interpretation difficult. These data could serve as a basis for further studies. Nevertheless, the studies deserve to be deepened to appreciate its chemical composition in fatty acids, the follow-up of its physicochemical indices during the storage in order to consider its use in various fields such as the agroalimentary or the pharmaceutical.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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