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Biometric Characterization of Fruits and Morphoanatomy of the Mesocarp of *Acrocomia* Species (Arecaceae)

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Abstract

The genus *Acrocomia* (Arecaceae) is widely distributed in the Neotropics, without consensus on the number of species. The arboreal species are explored in their native countries. To subsidize better use of the observed variation in fruits of different species for product extraction, taxonomy, conservation and genetic improvement, our objective was to characterize biometrically the fruits and anatomically the mesocarp from natural populations of *A. aculeata*, *A. intumescens* and *A. totai*. We observed different colors of epicarp and mesocarp in *A. aculeata* and *A. totai*, while the fruits of *A. intumescens* were light green or yellowish. The fruits of *A. aculeata* showed diameter considered large (3.9-4.6 cm) and the highest dry mass (26.3-33.5 g), *A. intumescens* presented intermediate diameter (3.1–3.9 cm) and mass (11.5-18.8 g), and *A. totai* had the smallest diameter (2.2-3.0 cm) and mass (4.1-11.4 g). The morphoanatomical analysis of the mesocarp did not distinguish the three species. However, it revealed the presence of useful compounds for human consumption and with economic potential, such as oil in the parenchyma cells, mucilage, starch and phenolic compounds. We concluded that the mesocarp anatomy is not useful for taxonomy, but the biometric variation is, as well as variation within species can be applicable in conservation and genetic improvement.

Keywords: biofuel, palm, plant anatomy, plant products, plant taxonomy

Resumo

O gênero *Acrocomia* (Arecaceae) é amplamente distribuído no continente americano não havendo consenso sobre o seu número de espécies, sendo as de porte arbóreo comercialmente exploradas nos países onde ocorrem. Com intuito de gerar subsídios para melhor aproveitar a variação observada nos frutos das diferentes espécies para taxonomia, conservação e melhoramento genético das espécies, o objetivo do presente estudo foi caracterizar biometricamente os frutos e anatomicamente o mesocarpo de populações naturais de *A. aculeata*, *A. intumescens* e *A. totai*. Foram observadas diferentes colorações de epicarpo e do mesocarpo em *A. aculeata* e *A. totai*, enquanto os frutos de *A. intumescens* são verde-claros ou amarelados. Os frutos de *A. aculeata* têm diâmetro considerado grande (3,9-4,6 cm) e de maior massa seca (26,3-33,5 g), *A. intumescens* apresentou diâmetro e massa intermediários dos seus frutos (3,1–3,9 cm e 11,5-18,8 g, respectivamente). *A. totai* apresentou os menores diâmetro e massa de frutos (2,2-3,0 cm e 4,1 -11,4 g, respectivamente). A análise morfoanatômica do mesocarpo não diferenciou as espécies do estudo. No entanto, revelou a presença de compostos úteis para consumo humano e potencial econômico dos compostos identificados, como a presença de óleo nas células do parênquima, mucilagem, amido e compostos fenólicos. Conclui-se que a anatomia do mesocarpo não é útil para taxonomia e que variação biométrica entre as espécies pode ser empregada para este fim, assim como a variação dentro das espécies pode ser útil para fins de conservação e melhoramento genético.

Palavras-chave: biocombustível, palmeira, anatomia, produtos vegetais, taxonomia

1. Introduction

The genus *Acrocomia* is Neotropical and the number of species is not yet well taxonomically well resolved. According to Henderson et al. (1995), only two species are attributed to the genus: *A. aculeata* (Jacq.) Lodd. ex Mart. and *A. hassleri* (Barb. Rodr.) W.J. Hahn, the first having large size, widely distributed along Central and South Americas, and the second, small, restrict to some areas of Cerrado of Brazil and part of Paraguay. Lorenzi et al. (2010) recognize seven species in the genus, six occurring in Brazil: *A. aculeata*, *A. intumescens* Drude and *A. totai* Mart., of arboreal size, and *A. hassleri, A. glaucescens* Lorenzi and *A. emensis* (Toledo) Lorenzi, of small size; and *A. crispa* (Kunth) C.F. Baker ex Becc., of arboreal size, endemic to Cuba. Furthermore, according to The Plant List (2013), all previously cited species are considered valid plus *A. media* O.F. Cook., endemic to Porto Rico and Virgin Isles, also of arboreal size, but Proctor (2005) mentions it as a lower plant, with larger leaves and smaller spathe compared to *A. aculeata*.

The species of *Acrocomia* are utilized for several purposes. The palms are utilized as ornamental species in different countries of occurrence (Lima, 1994; Moraes, 2004; Silva, 2012). In Mexico, Bolivia and Brazil the roots are utilized as medicine (Amorozo & Gély, 1988; Hernández et al., 2011; Moraes, 2004). In countries of Central America, Mexico, Venezuela and Brazil it is common the utilization of sap of the stipe to prepare an alcoholic drink (Balick, 1984; Bran, 2013; Corrêa, 1984; Hernández et al., 2011; Lentz, 1990; Plotkin & Balick, 1984). The leaves are utilized for rooves or as raw material to obtain fibers for production of lines, ropes and nets, and as good quality forage (Moraes, 2004; Pott, 1986).

Although all parts are utilized, the fruits have the highest diversity of economic exploration, being utilized for human fresh or processed food, presenting good nutritional quality and medicinal purposes (Hernández et al., 2011; Ramos et al., 2008). Nevertheless, the highest interest in exploration of the fruits is for their potential for production of oil of the mesocarp (pulp) for biofuel, over 4,000 Kg/ha/yr, and of the nut oil for cosmetics.

Among the recognized species, in Brazil, three are explored for extractivism for presenting fruits of commercial interest: *A. aculeata*, popularly known as macaúba, with occurrence in various states of Brazil; *A. intumescens*, locally called macaíba, endemic to the Northeast region, occurring in areas of the called *Zona da Mata* (forest zone)and in altitude forests; and *A. totai*, known as bocaiúva, distributed in most part of the state of Mato Grosso do Sul, associated to areas de Cerrado and Pantanal (Lorenzi et al., 2010; The Plant List, 2013).

The three species can be morphologically distinguished, mainly by the characteristics of the stipe: *A. aculeata* has very spinescent stipe and presence of the rests of leaf sheath; *A. totai* presents lower number of spines and sporadically the rests of the leaf sheath; and *A. intumescens* has spines only when young, presenting swelling in the middle of the stipe and without rests of the leaf sheath (Lorenzi et al., 2010).

The fruit is of the drupe type, derived from superior ovary, characterized by the presence of stony epicarp (peel), fleshy mesocarp (pulp) and stony endocarp (Esau, 1977). The fruits of the species of *Acrocomia* are globose, present hard epicarp, strongly adhered to the mesocarp when young, and generally brittle, easily detaching from the mesocarp when ripe. The mesocarp is mucilaginous, with large quantity of fibers. The endocarp is woody and thick (Dransfield et al., 2008; Lorenzi et al., 2010).

The three studied species are explored for consumption or commercially. The fruits of *A. intumescens* in the Northeast region and the fruits of *A. totai* in the state of Mato Grosso do Sul are explored by local communities for consumption of pulp and fresh or processed nut in several types of foods, such as meal, ice cream, cake and others. Some of the products are sold by these communities as source or complement of family income. The species *A. aculeata* has been explored, mainly in the state of Minas Gerais, for extraction of oil of pulp and nut for production of biofuel and cosmetics. Historically, the oil of *A. aculeata* was used for street lightning of important old mining towns, such as Ouro Preto and Mariana, Minas Gerais.

Although *A. aculeata* nowadays is the species most utilized for production of biofuels, containing between 37 and 78% of oil in the mesocarp (Berton, 2013; Conceição et al., 2012), the other species also present potential for oil production. *Acrocomia intumescens* presents between 34 and 41% of oil in the mesocarp (Bora and Rocha 2004; Conceição et al. 2012). The species *A. totai* presents oil content in the mesocarp between 14 and 31% (Ciconini et al., 2013; Conceição et al., 2012; Hiane et al., 2005).

Considering that the economic potential of the species of *Acrocomia* is based, mainly, in the exploration of its fruits, studies on biometric characterization of fruits of different species from different regions are of great importance. The biometric characterization of fruits is fundamental to subsidize conservation and exploration of plant resources, since such data allow to estimate the productivity and to sort more homogeneous seed lots and, consequently, with more uniformity and vigor (Moura et al., 2010). It is also a useful tool for detection of genetic variability within populations of a same species and the relations between this variability and environmental factors, information useful in genetic programs (Carvalho et al., 2003). The tropical arboreal species present striking differences regarding fruit size, number and size of seeds (Silva et al., 2007), so the biometry of fruits can be useful for taxonomic distinction, as doubts persist yet upon the most adequate number of species for the genus *Acrocomia*.

However, most botanical classifications are based only on morphological data, many of them presenting variation of genotype *vs.* environment nature. So, mistakes may occur, what demonstrates the importance of utilization of other techniques which could help to split species. The study of fruit anatomy is a valuable tool for the classification of the type of fruits and, consequently, for Taxonomy (Souza et al., 2012), for practically not varying under environmental interference, and can also provide valuable data for identification of plant products (Vaughan, 1960). The histochemical detection of substances in fruits is of great importance not only for taxonomy but also for identification of active principles and other substances which can be utilized by the industry (Dôres, 2007). The commercial value of the fruits and the types of industrial processes needed, for example, for extraction of oil are determined by the nature and anatomical structure of fruits (CETEC, 1983).

The studies of fruits involving species of *Acrocomia* are yet scarce. So, our work was carried out with the objective to characterize biometrically fruits of the three arboreal species of *Acrocomia* of highest commercial interest with occurrence in Brazil and with great potential for utilization in the other countries where they occur, as well as to characterize anatomically the mesocarp to verify which tissue and or structure of the mesocarp contains the oil and if it is possible to perform the quantification of such cells and or structures aiming its adoption as selection criterion for purposes of genetic improvement.

2. Method

2.1 Sampling Area and Fruit Biometry

The chosen species of *Acrocomia* are in accordance with Lorenzi et al. (2010). The individuals selected for our study are from different regions of Brazil. The individuals of *A. aculeata* from two natural populations in a transition area between Cerrado and Mata Atlantic forest in the municipality of Itapira, São Paulo. The individuals of *A. intumescens* were collected from a population in an area of Atlantic forest in the metropolitan region of Recife, Pernambuco, and the plants of *A. totai* were selected from two native populations of the Pantanal in the municipality of Corumbá, Mato Grosso do Sul. We sampled 30 fruits at random from ten individuals of each population (Figure 1). According to Manfio et al. (2011), evaluation of four fruits in a population is sufficient to assess the studied characteristics.

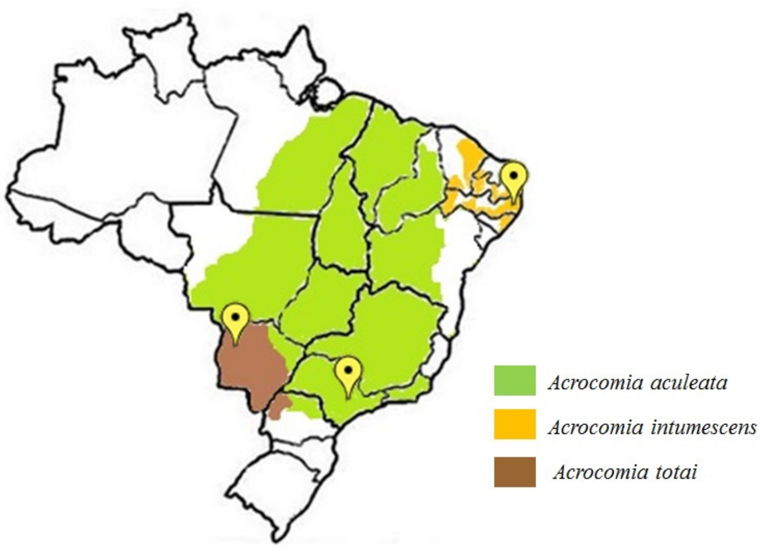


Figure 1. Area of occurrence and collection points of the three studied species of *Acrocomia* in Brazil

The fruits were measured as for their external longitudinal diameter (length), external transversal diameter (diameter) and individually weighed for determination of fresh mass and dry mass given by drying in oven with air circulation at 58°C, until constant weight. The percentage of moisture in the pulp was obtained by difference between fresh mass and dry mass, divided by fresh mass (Moura, 2010). We calculated the length/diameter ratio (C/D) of the fruits, regarding that the ratio C/D < 1 indicates flat shape, C/D > 1 is ovoid, and the ratio C/D = 1 means globose (Sacramento et al., 2003). The obtained data were analyzed by univariate statistics, covering measures of position (medium, minimum and maximum) and of dispersion (standard deviation, coefficient of variation), as well as distribution of frequency. We performed analysis of variance (ANOVA) with Tukey's post-test, at 5% of probability.

The biometric characteristics of fruits of the studied species of *Acrocomia* were compared as for their diagnostic potential for taxonomy and genetic improvement, using groupment analysis. We analyzed the four main characteristics related to productivity: external longitudinal diameter, external transversal diameter, and fresh and dry masses. The data were distributed in classes and then tabulated in form of binary matrix described as presence (1) or absence (0) and then submitted to groupament analysis utilizing the algorithm UPGMA and the Euclidian distance as measure of similarity among sampling units using the software PAST (Hammer et al., 2001). The stability dos groupments was tested applying the procedure of resampling by 10000 *bootstraps*.

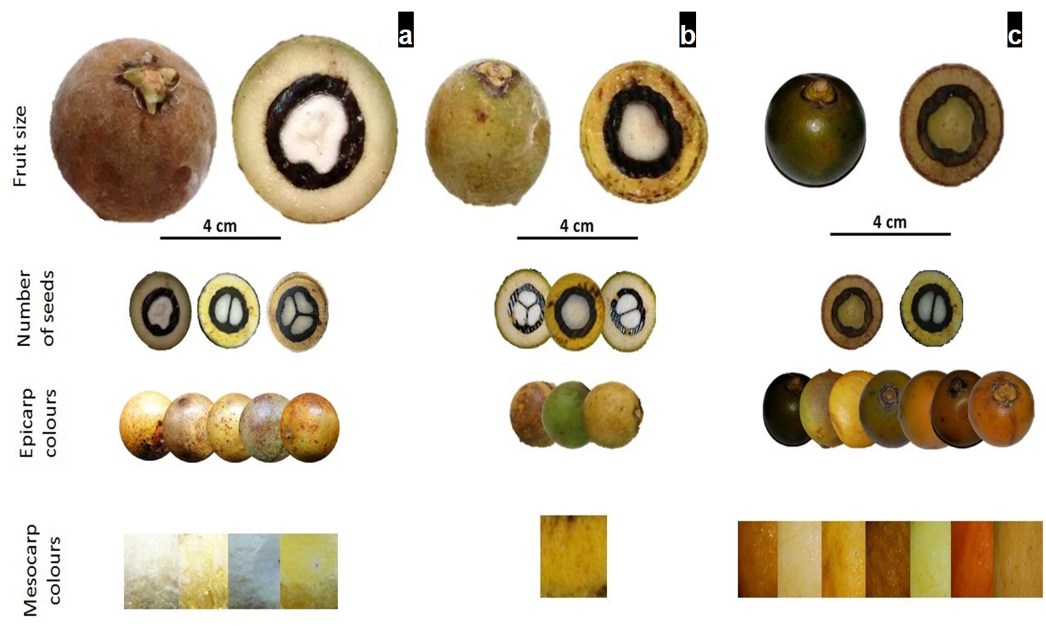
2.2 Morphoanatomy of the Mesocarp

Three fruits of each population were collected for analyses of anatomy, fixed with FAA_{70} for 48 hours and stored in ethylic alcohol 70%. Next, we removed a small piece of the central region of the fruit with a band-saw and then dehydrated it in ethylic series. Afterwards, the material underwent a pre-infiltration in different concentrations of a solution of synthetic resin (Historesin ® Leica) and alcohol and then immersed in pure synthetic resin, sectioned in rotative microtome and stained with toluidine blue 0.05% in acetate buffer 0.1 M (pH 4.7) (O'Brien et al., 1964). We performed histochemical tests utilizing Sudan IV for detection of total fats (Pearse 1985), Lugol for detection of starch (Gerlach, 1984), Ruthenium red (Jensen, 1962) for detection of pectic substances, polysaccharides and acid mucilage and iron III chloride (Johansen, 1940) for detection of simple phenols. The documentation of the slides set of the optic microscopy was obtained under photomicroscope Olympus BX 51 with system and coupled image capture model DP71.

3. Results and Discussion

3.1 Biometric Characterization of Fruits

The fruits of *A. aculeata* and *A. totai* present different peel and pulp colors, even in individuals of the same population. The fruits of *A. intumescens* presented lower variation and are generally light green or yellowish when ripe. It is also possible to perceive visually the contrast of size among fruits of the different species (Figure 2).



Photos *Acrocomia aculeata* : Berton, L.H.C.

Figure 2. Morphological variation of number of seeds, color of peel and color of pulp of: **a** *Acrocomia aculeata*, **b** *A. intumescens* and **c** *A. totai*.

To demonstrate graphically the variability of characteristics within and between the studied species of *Acrocomia*, each measured characteristic was evaluated considering the data of the three species altogether and then determined classes of distribution of data. The data of absolute frequency are represented in the graphs by the columns and the relative frequency (%) of data distribution of each characteristic within each species is represented by lines (Figure 3).

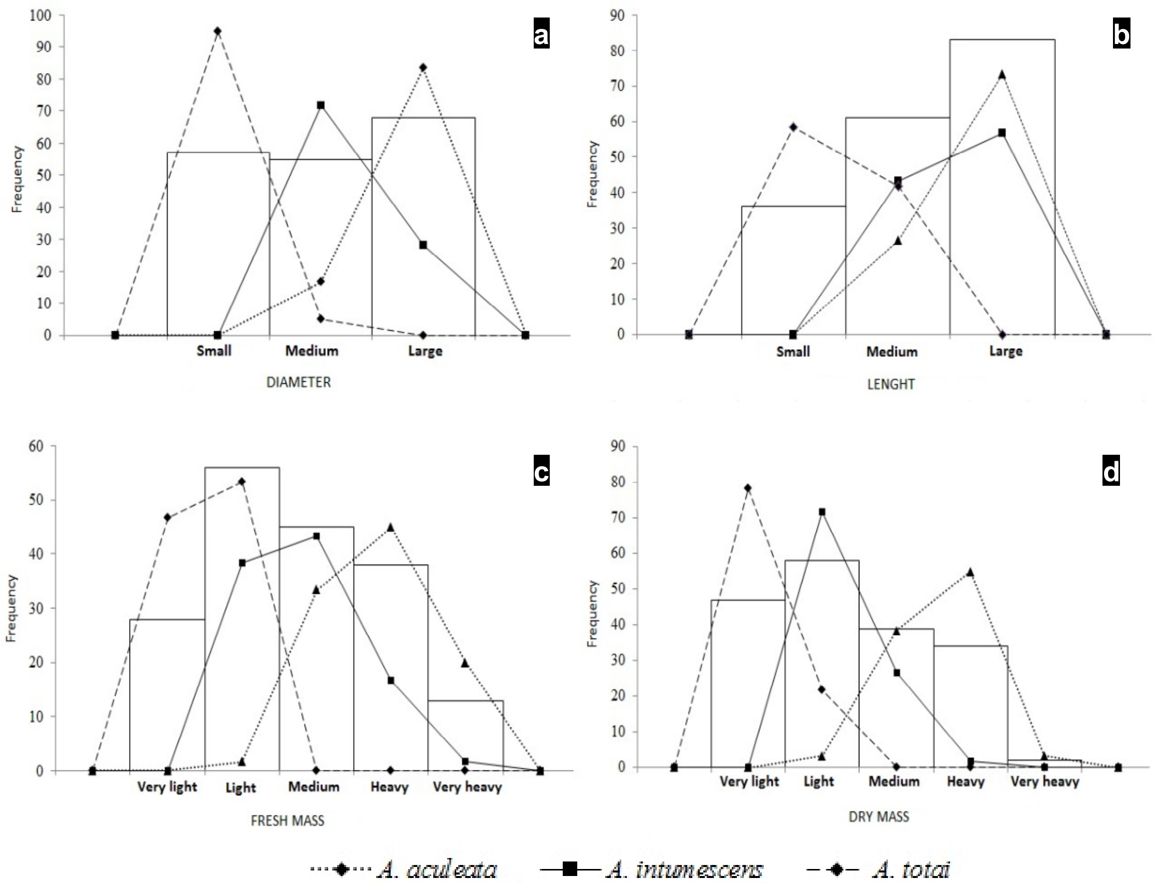


Figura 3. Distribution of classes of data of diameter, length, fresh mass and dry mass of *Acrocomia aculeata*, *A. intumescens* and *A. totai*.

Regarding diameter, most sampled fruits belong to the class "large", between 4.0 and 4.7 cm. Although occurring overlap of part of the values in some points of the distribution, the separation of peaks is quite clear, i.e., of the highest frequency of each biometric characteristic within species in the distribution. Most fruits of *A. aculeata* (83.33%) have diameter considered large, *A. intumescens* most fruits (71.67%) with intermediate diameter (3.1 – 3.9 cm) and *A. totai* has most fruits (95%) with small diameter, between 2.2 and 3.0 cm (Figure 3 a).

Concerning length, most part of data was concentrated in class "large", measuring 3.9 to 4.6 cm. As can be observed in Figure 3 b, there was data overlap of *A. intumescens* and *A. totai*, with great part of their fruits with "medium" size (3.0 to 3.8 cm). However, the highest frequency of fruit length in *A. totai* (58.33%) was concentrated in the class "small" with fruits measuring between 2.1 and 2.9 cm, and *A. aculeata* and *A. intumescens* with most fruits (73.33 and 56.67%, respectively) distributed in class "large".

Most sampled fruits, considering the three species together, presented fresh mass between 16.5 and 26.2 g, being represented in the class "light". There was overlap of part of the fruits of *A. aculeata* and *A. intumescens* (43.33%) in the class of fruits with fresh mass "medium", with weight between 26.3 and 35.9 g. In *A. totai* the fruits were distributed between the classes "very light" (6.9 – 16.4 g) and "light", most part concentrated in the class "light" (53.33%). Most data of fresh mass of *A. aculeata* was concentrated in class "heavy" (45%) with fruits weighing between 40 and 45.5 g (Figure 3 c). In spite of the observed overlap of data of dry mass in the distribution of classes, the formation of three peaks is well evident, each one indicating the highest frequency of data of dry mass determined within each species (Figure 3 d). *Acrocomia aculeata* has most fruits in the class "heavy" (55%), with dry weight between 26.3 and 33.5 g. *Acrocomia intumescens* has the highest frequency of fruits (71.67%) with weight between 11.5 and 18.8 g concentrated in class "light", and *A. totai* presents most fruits in the class "very light" (78.33%), weighing between 4.1 and 11.4 g.

The morphological diversity both within and between analyzed species of *Acrocomia* was with utilization of the distribution in classes of the evaluated biometric characteristics. The variability within each species was explained by the presence of fruits in different classes of each characteristic. For example, *A. aculeata* has fruits distributed into four of the five classes established for fresh mass (Figure 3 c). The variability among species graphically demonstrated by the peaks of distribution of the biometric characteristics within each species, such as the diameter classes forming three peaks, each peak representing one well defined species, each one covering a certain class of distribution (Figure 3 a).

The fruits of *A. aculeata* showed the largest mean diameter and length (4.13 ± 0.27 cm and 4.01 ± 0.29 cm, respectively), followed by *A. intumescens* (3.69 ± 0.29 cm and 3.88 ± 0.27 cm, respectively) and *A. totai* with the lowest mean fruit size, 2.72 ± 0.24 cm diameter and 2.84 ± 0.30 cm length (Table 1).

The highest mean fresh mass of the fruit was also found in individuals of *A. aculeata* (39.04 ± 7.06 g). *Acrocomia intumescens* has mean fresh mass of 29.08 ± 6.87 g, and *A. totai*, the lowest mean value of fresh mass (16.39 ± 4.16 g), compared to the other studied species. The highest mean dry mass value also occurs for *A. aculeata* (26.69 ± 4.54 g), intermediate value in *A. intumescens* (17.27 ± 3.33 g) and the lowest dry mass in *A. totai* (9,42 ± 2,83 g) (Table 1).

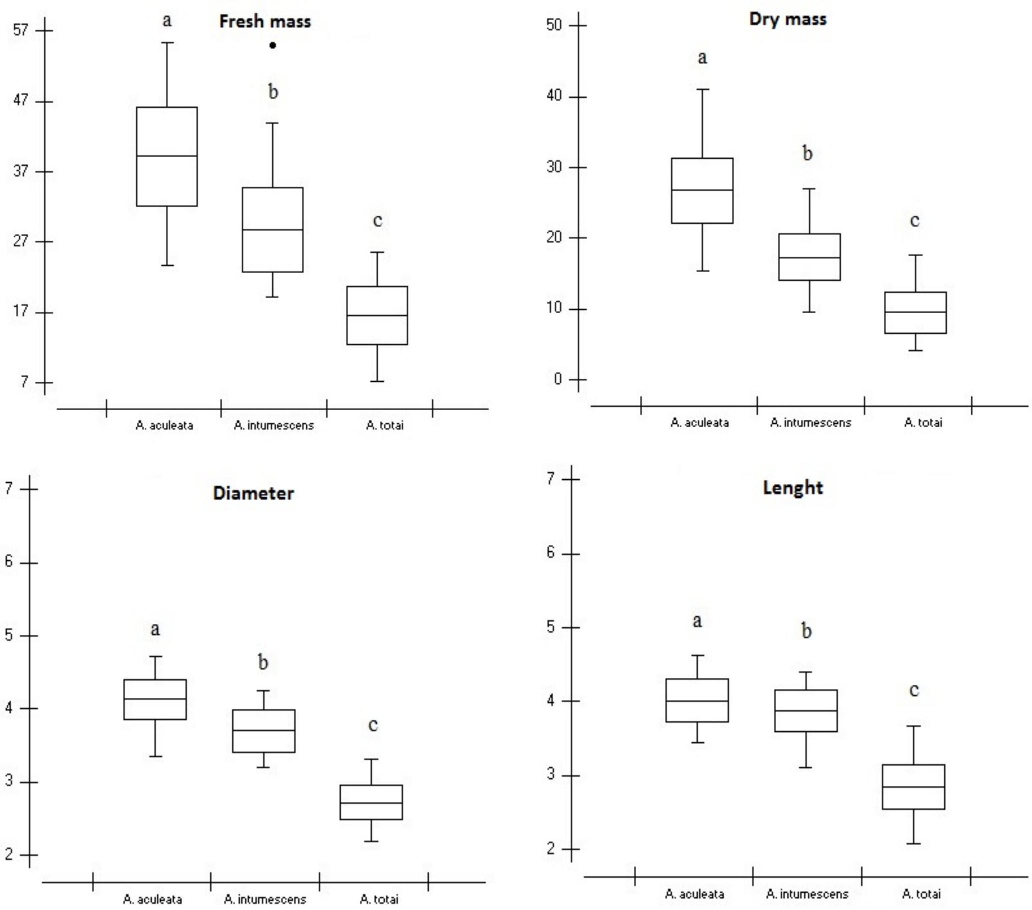
Table 1. Biometric characteristics of fruits of *Acrocomia aculeata*, *A. intumescens* and *A. totai*: lower limit (LL), upper limit (UL), mean, standard deviation (σ) and coefficient of variation (CV%)

	LL	UL	Mean	σ	CV%
Acrocomia aculeata					
External longitudinal diameter (cm)	3.44	4.62	4.01	0.29	7.28
External transversal diameter (cm)	3.35	4.72	4.13	0.27	6.50
Fresh mass of the whole fruit (g)	23.55	55.16	39.04	7.06	18.09
Dry mass of the whole fruit (g)	14.34	40.91	26.69	4.54	17.00
Moisture of the fruit (%)	8.96	44.30	31.05	8.49	27.34
Ratio C/D	0.83	1.09	0.97	0.05	4.67
Acrocomia intumescens					
External longitudinal diameter (cm)	3.10	4.40	3.88	0.27	7.08
External transversal diameter (cm)	3.20	4.25	3.69	0.29	7.86
Fresh mass of the whole fruit (g)	1894	54.92	29.08	6.87	23.61
Dry mass of the whole fruit (g)	9.49	27.00	17.27	3.33	19.29
Moisture of the fruit (%)	2870	73.82	39.62	7.30	18.43
Ratio C/D	0.95	1.26	1.05	0.06	5.52
Acrocomia totai					
External longitudinal diameter (cm)	2.07	3.65	2.84	0.30	10.66
External transversal diameter (cm)	2.17	3.30	2.72	0.24	8.90
Fresh mass of the whole fruit (g)	6.89	25.40	16.39	4.16	25.39
Dry mass of the whole fruit (g)	4.10	17.57	9.42	2.83	30.06
Moisture of the fruit (%)	21.05	65.04	42.53	7.79	18.32
Ratio C/D	0.74	1.43	1.05	0.10	9.41

Acrocomia aculeata presents ratio C/D of 0.97 and *A. intumescens* and *A. totai* have 1.05, i.e., the fruits of the three studied species present ratio C/D close to 1, evidencing globose shape (Table 1).

Moisture content of fruits also varied among species, *A. totai* presenting the highest content (42.53 ± 7.79%), followed by *A. intumescens* (39.62 ± 7.30%) and the lowest in fruits de *A. aculeata* (31.05 ± 8.49%) (Table 1). The values can vary according to the ripening stage of fruits, form of harvest and preservation time.

Significant differences ($p < 0.05$) were detected among the means of length, diameter, fresh mass and dry mass of fruits, i.e., the obtained results rejected the hypothesis of equality among means, demonstrating the existence of biometric variation among species, *A. aculeata* standing out for the highest values for all cited biometric characteristics, *A. totai*, the lowest values and *A. intumescens* intermediate values (Figure 4).



* Same letters do not differ statistically at 5% probability (p < 0, 05) test de Tukey.

Figure 4. Biometric characteristics of fruits of *Acrocomia aculeata*, *A. intumescens* and *A. totai*.

The four main biometric characteristics of fruits (diameter, length, fresh and dry mass) utilized for groupment analysis resulted in separation of the studied species of *Acrocomia*, as illustrated in Figure 5.

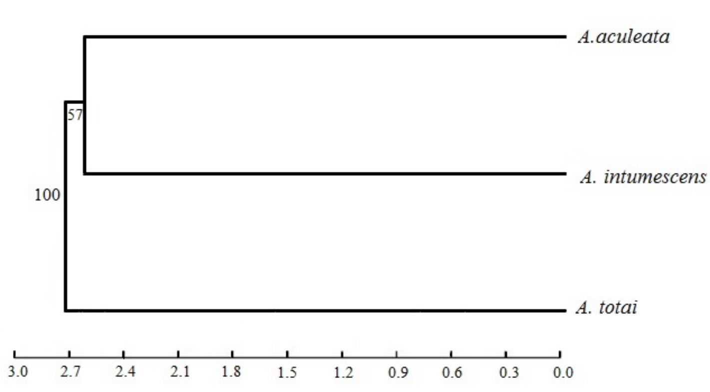


Figure 5. Groupment analysis obtained by Euclidian distance and UPGMA, based on the characteristics diameter, length, fresh and dry masses of fruits of the three studied species of *Acrocomia*.

The analysis resulted in closeness of *A. aculeata* and *A. intumescens* and separation of *A. totai* from both, what can be explained by the values remarkably lower of all evaluated biometric characteristics found in *A. totai*. Figure 3 illustrates that *A. totai* presents the peaks with highest frequencies of the samples of each evaluated characteristic well separated from the other species, and that *A. aculeata* and *A. intumescens* present some peaks of highest frequency of similar data, as well as overlap of samples in a same class, e.g. frequency distribution of length data (Figure 3 b), where *A. aculeata* and *A. totai* have the highest frequency in the same class "large".

The fruit biometry data support the classification of *Acrocomia* proposed by Lorenzi et al. (2010). The main morphological characteristics which distinguish the studied species of *Acrocomia* are summarized in Table 2 (Figure 6).

Table 2. Summary of the morphological characteristics of three species of *Acrocomia*.

sp.	Size	Stipe	Leaf length (m)	Spines	Fruit color	Fruit diameter (cm)	Habitat
Aa	Arboreal	Cylindric, straight, with remnants of base of dropped leaves	1.9-3.0	Stipe and leaves	Varied	3.0-5.0	In general dry and open areas
Ai	Arboreal	Cylindric, swollen close to the middle, smooth	2.4-3.0	Stipe of young plants	Greenish, light yellow	3.1-4.5	Atlantic forest and Altitude forest
At	Arboreal	Cylindric, straight, without remnants of base of dropped leaves	2.0-2.6	May occur or not on stipe and leaves	Varied	2.3-4.3	Dry and open areas

* **Aa:** *Acrocomia aculeata*, **Ai**: *Acrocomia intumescens* and **At**: *Acrocomia totai*. The described data result from our observations and from the revision of *Acrocomia* by Lorenzi et al. (2010).

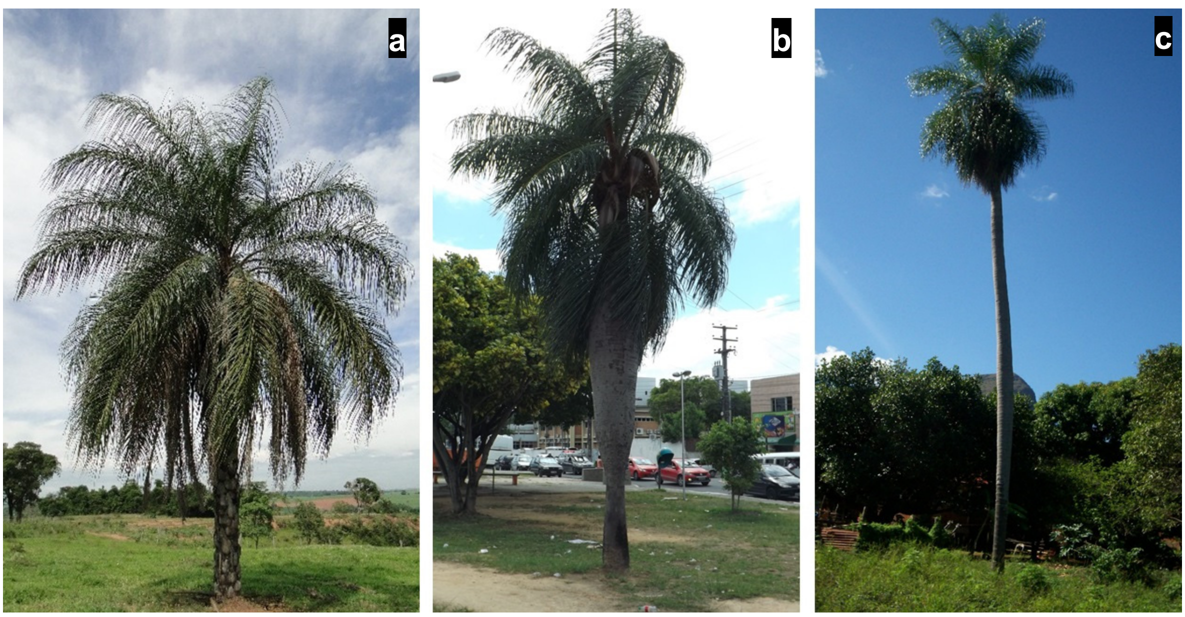


Figure 6. Adult individuals of **a** *Acrocomia aculeata*, **b** *A. intumescens* and **c** *A. totai*.

3.2 Anatomy of the Mesocarp

The analyzed species of *Acrocomia* present similar morphoanatomy of the mesocarp, not representing a useful character for taxonomy of the genus (Figure 7 a-c).

The mesocarp is formed in most part by parenchymatic cells. Throughout the mesocarp can be observed large rounded cavities, with thin walls, irregularly distributed and frequently fused forming large spaces with irregular shapes (Figure 7 a-c). With the performed tests it was not possible to prove if these cavities (Figure 7 e, f) are the responsible for the high production of mucilage of the fruits, since the test for polysaccharides (Ruthenium red) was not positive.

All over the mesocarp are also found bundles of non-vascular fibers and vascular bundles, the latter being involved by sclerenchymatic sheath. The vascular bundles are of similar size and are irregularly distributed throughout the mesocarp (Figure 7 d).

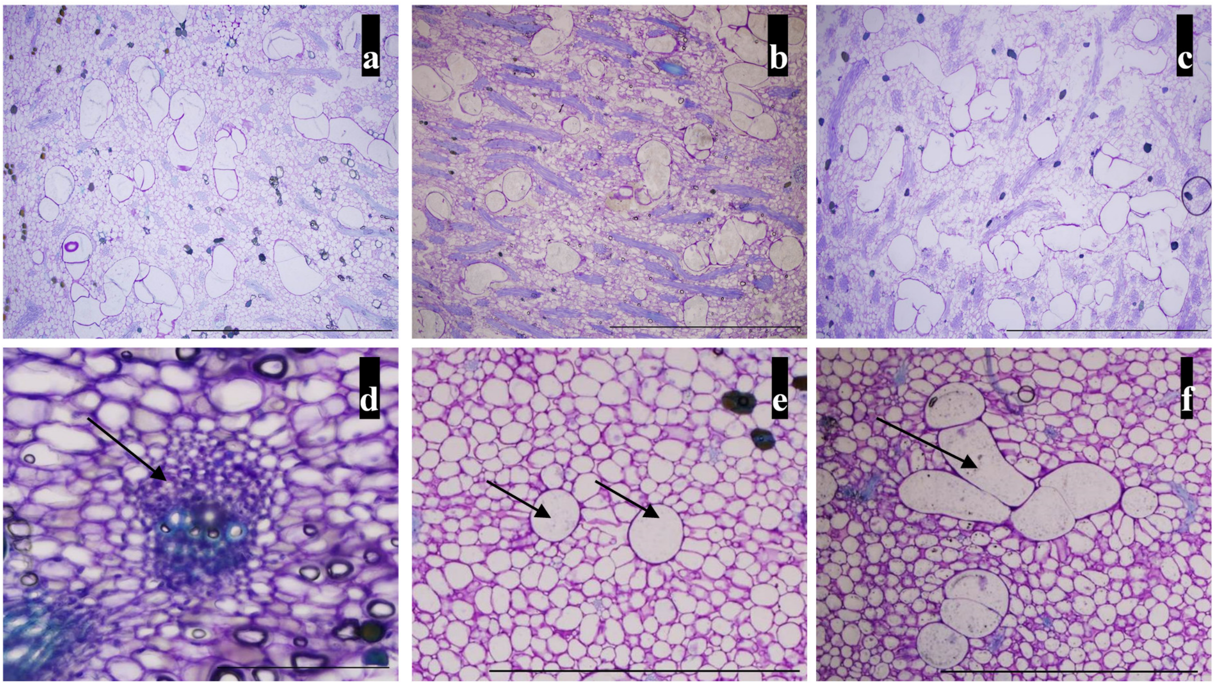


Figure 7. Section of mesocarp: **a** *Acrocomia aculeata*, **b** *A. intumescens*, **c** *A. totai*, **d** detail of vascular bundle, **e** cavities with mucilage and **f** cavities with mucilage fused. Bar: 1mm (a-c), 100 μm (d), 500 μm (e, f).

3.3 Histochemical Tests

The performed histochemical tests were also similar as for presence and quantity of the tested compounds, only differing in starch content, detected in highest quantity in *A. intumescens*, followed by *A. aculeata*, but absent in *A. totai*, and the highest presence of phenolic compounds in *A. aculeata* and *A. totai.* The assessed compounds and their intensity are summarized in Table 3.

Table 3. Detected compounds in the mesocarp in the three species of *Acrocomia.*

Species	Total fats	Starch	Total polysaccharides	Simple phenols
Acrocomia aculeata	+ +	+ / -	-	+
Acrocomia intumescens	+ +	+	-	+ / -
Acrocomia totai	+ +	-	-	+

+ + Great quantity; **-** Not observed; **+ / -** Observed in some individuals and not in others.

3.3.1. Total Fats

Practically all parenchymatic cells of the mesocarp in all studied species contain oil as evidenced by positive reaction to Sudan IV, so, all species present potential for extraction. Oil occurs in parenchymatic cells of the mesocarp in form of lipid bodies, also called oleosomes or lipid droplets, which can present varied sizes depending on the species and or stage of the studied fruit parts. These organelles synthesize and store triacilglycerols (TAGs), associated to proteins that delimit, i.e., compartimentamentalise the organelle, being responsible for the temporary storage and efficient of carbon of high energy (Horn et al., 2013, Ho et al., 2014).

In case of this work, the oleosomes could not be observed in sections in the mesocarp, probably due to immersion process of the material in synthetic resin. In hand cuts made on material only fixed was possible to observe such organelles, however, due to the large quantity and overlap of oleosomes present, it was not possible to see the parenchymatic cells individually as it was also not possible to count the oleosomes (Figure 8 a, b). However, if altering the immersion technique of the material and the type of microscopy is possible to quantify and even measure the size of oleosomes. Ho et al. (2014) made quantification and measurement of oleosomes present in the embryo, in the mesocarp and in the nut of the oil palm (*Elaeis guineensis* Jacq.), finding differences between quantity and size of such organelles depending on the tissue and its development stage, however, utilizing other fixation and inclusion techniques and with use of transmission scanning microscope (Figure 9).

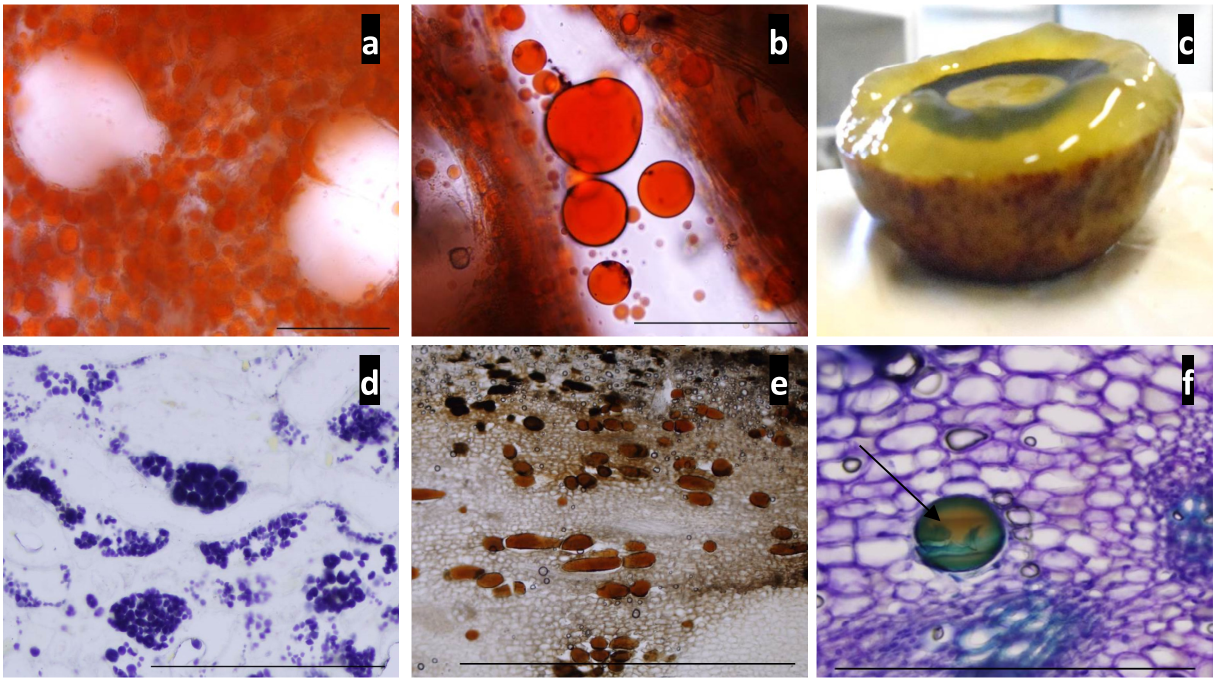


Figure 8. Section of mesocarp in *Acrocomia* spp.: **a** oleossomes in the mesocarp, **b** detail of oleossome, **c** mucilage, **d** starch grains, **e** phenolic compounds detected with Ferric Chloride III, **f** cell with phenolic compound in detail, stained with toluidine blue. Bar: 100 µm (a, b, d, e), 1 mm (f)

3.3.2 Mucilage

Although not having being visualized with the use of Ruthenium red, the quantity of mucilage present in fruits of *Acrocomia* is very high. Although the mucilage is concentrated in large cavities in the mesocarp, due to its great quantity it can permeate the epicarp, what was proven by visualization of the mucilage in the pulp because the alcohol in which fruits were preserved became very viscose (Figure 8 c).

Mucilage is a macromolecular, complex carbohydrate, of varied composition (acid or neutral polysaccharides) according to the species, with hydrophilic properties (Sáenz et al., 2004). Due to its great capacity to absorb water, in whose presence it becomes viscose, acts in plants as a water reservoir (Cárdenas et al., 1998), having important physiological functions, such as reduction of transpiration in dry climates, water and nutritive substance storage, and protection against pests and pathogens (Alvarez et al., 1992; Fahn, 1979; Rocha et al., 2011). The production of mucilage in great quantity in fruits of *Acrocomia* spp. can suggest its role as adaptation to dry climates, where they are most frequent, or as chemical characteristic of species of the genus. Rocha et al. (2002), studying species of *Hibiscus* (Malvaceae) in xeric habitat, and Colonetti (2012), working on a cactus, verified the role of mucilage as storage substance and water retention. Yet, Silva and Potiguara (2009), analyzing the leaf histochemistry of *Oenocarpus* species (Arecaceae) from Amazonia, attributed the occurrence of mucilage, not to an ecophysiological adaptation, but to a probable characteristic of that palm group.

Since it was not possible to detect mucilage with the use of Ruthenium red in samples of our study, utilized for detection of total polysaccharides and acid mucilage (Jensen, 1962), we suppose that the mucilage present in the mesocarp of the studied species of *Acrocomia* is predominantly neutral. Gregory and Baas (1989, *apud* Rocha et al., 2011) suggested that different types of mucilage exert distinct functions, where water storage would be made by the acid fraction, with production peak during the Summer months, and the neutral fraction of the mucilage would be responsible for build-up of carbohydrates, with peak during Winter. Such finding supports the hypothesis of the presence of mucilage in the mesocarp of *Acrocomia* species exerting the function of water storage and retention. This can be even more feasible considering biochemical and anatomical studies on *A. aculeata* fruit development, with peak of mucilage accumulation in the mesocarp until natural fruit abscission (Montoya, 2013, Reis et al., 2012). And we point out that the abscission phase of fruits of species of the genus, indicating ripeness, occurs during the warmer months in Brazil.

It is worth to highlight that mucilages, due to their viscose consistency in presence of water, have sticky and thickening properties, much utilized by the food industry in sweets and jams, and by the pharmaceutical industry for stability of emulsions and ointments, plus acting as dietetic fiber in the human organism promoting reduction of cholesterol, control of glycose, reduction of the risk of some types of cancer and of the symptoms of chronic constipation and hemorrhoids (Colonetti, 2012). I.e., the fruits of the species of *Acrocomia*, beside its use for oil extraction, due to the large quantity of mucilage present in the mesocarp can also be extracted and sold to different industrial segments. Furthermore, the process of demucilage, i.e., the extraction of mucilage from the plant tissue, can increase oil yield, as demonstrated by Speroni et al. (2015), who increased the efficiency of oil extraction by 30% after demucilage of flax seeds (*Linum usitatissimum* L.).

3.3.3 Starch

The Lugol test was only positive for *A. intumescens* and one individual of *A. aculeata*. Starch in these species was observed in the parenchymatic cells of the mesocarp. The amyloplasts are grouped in spheres, what was also observed by Bonin et al. (2008) in samples of *A. aculeata* from another region of the state of São Paulo (Figure 8 d).

Starch are natural polysaccharides, little soluble and of high molecular weight, formed by various sequences of amylose and amylopectin. Amylose is a linear polymer, and amylopectin is a well ramified macromolecule, spheric to elliptic (Salisbury & Ross, 1992). The shape of starch grains can be distinguished mainly by the quantity of amylose, since the higher the content of amylose in relation to amylopectin, more spheric are the starch grains (Bewley & Black, 1994). So, due to the spheric shape of the starch grains present in the studied species of *Acrocomia*, we infer that the quantities of amylose are higher, similar to the report by Silva and Potiguara (2009) who also found spheric starch grains in species of *Oenocarpus* (Arecaceae). This corroborates Tomlinson (1961) about the lack of morphological diversity of starch grains in Arecaceae, therefore it is not a useful character for taxonomic distinction in this botanical family.

According to Montoya (2013) and Reis (2012), the accumulation of starch in fruits of *A. aculeata* happens during all development phases, after fecundation of the ovule. Nevertheless, after the 36th week post-anthesis there is a marked reduction of starch content and thereafter a linear increase in production and accumulation of oil, so presenting a relation between the degradation of starch and the synthesis of fatty acids after this phase (Montoya 2013), what is explained by the fact that the synthesis and storage of oil in plant tissues is directly related to the availability of stored polysaccharides, e.g. starch (França et al. 1999). Thus, we suggest that the fruits where starch was not detected would be in a more advanced phase, when the stored starch had already been converted into oil in the mesocarp.

3.3.4 Phenolic Compounds

We observed cells with phenolic compounds in all analyzed species. According to Santos (2012), the phenolic compounds present in the mesocarp of *A. aculeata* occur inside the idioblasts. The largest quantity of phenolic idioblasts occurred among the analyzed samples of *A. aculeata* and *A. totai* (Table 3 and Figure 8 e, f). Phenolic compounds have antimicrobial activity and exert action against herbivory (Rocha et al., 2011b; Swain, 1959).

Regarding economic potential, the phenolic compounds are the most abundant antioxidants in plants, because they can mitigate cumulative harms that can trigger several diseases (Rocha et al., 2013). *Acrocomia aculeata* stands out for its content of total phenolic compounds (Aragão, 2014; Rocha et al., 2013; Siqueira, 2012), supporting its potential use as fresh food or processed functional food. All detected compounds, beside oil, are liable to utilization by different industrial segments.

The description of the morphoanatomy of the mesocarp and the detection of the main biochemical compounds represent an important contribution upon fruits of Arecaceae, as there are not enough studies on structural organization of the vegetative organs and, moreover, of the reproductive organs. Ontogenetic studies of fruits of the other species of *Acrocomia* and of *A. aculeata* of different localities are needed to understand origin, function and organization of each tissue present in the fruits. Such studies could generate data which may could serve for taxonomic purposes, beside subsidize knowledge necessary for management for extraction of any fruit compound, for seed production and plant breeding.

The histochemical tests, in addition to demonstrate that the high oil content present in other species than *A. aculeata* can also be utilized for extraction, evidenced the presence of other compounds, such as starch, phenolic compounds and mucilage that can contribute not only in nutrition as well as raw material for different industrial segments.

4. Conclusions

The evaluation of biometry of fruits revealed the existence of morphological variation within and between populations and among the three species of *Acrocomia aculeata*, *A. intumescens* and *A. totai*, complementing data of plant morphology as well as confirming the hypothesis that the species are distinct. The morphoanatomy of the mesocarp of the analyzed species is similar. The mucilage in the mesocarp is responsible for water storage. Starch is stored until the fruit begins to ripen, when it is converted into oil. The oil present in the mesocarp occurs inside oleosomes.

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