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Physical and Morphological Characteristics of the Amazon Palm Berry Assai (*EuterpeOleracea*Mart.) – Estereoscopy

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Abstract:*The assai (in Portuguese – açai) berry (Euterpeoleracea Mart.) morphological characteristics of skin, fleshand seed were investigated by stereo microscopy including dimensions (height, width, depth), shape and parts proportion (skin /flesh/ seed). Samples were from 2 assai producers (PI and PII) Amazon Eastern region (Northern Brazil).* ***Assai morphological*** *characteristics* ***(a) skin: outer surface*** *– a dark purple to bordeaux smooth layer with hilum, germination pore (opposite to each other);****inner surface****- polygonal cells with fibers at the hilum region;* ***(b) flesh: pulp& fibers****-tinny layer with cells full of hydrophilic and lipidic nutrients, surrounded by mesocarpicfibers and* ***(c) seed:*** *a quite hard structure that occupies most of the whole berry (seed coat + endosperm).Assai proportion between skin, flesh and seeds was 82.08-3.6% (seed) and16.2-17.2% (skin + flesh altogether).*

Key words: *Assai, acai, Euterpeoleracea, pulp, microscopy, stereoscopy, amazon.*

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I. Introduction

The assai (*Euterpeoleracea* Mart.), in Portuguese *açaí*, is a Brazilian palm berry fruit grown mainly in the Amazon forest (Northern region) and *Cerrado* (Northeastern region) (Jardim et al. 1987; Nogueira et al. 1995; Arruda et al. 2014). It has a quite high economic importance to those regions and country (Rhoma et al. 1987; Arruda et al. 2014). Its frozen pulp is the best-known commercial available food product (Dominici, 2016).

The consumption of assai fruit and its products has increased in recent years in all Brazilian regions although the main consumption is still in the Northern region (Rogez et al. 1996, 2011; Cialdella et al. 2014). The same occurred for export, mainly to the United States and European Union (Embrapa, 2006; Sabbe, 2009; De Lima et al. 2015).

Assai is consumed in different ways depending on the region. In the Northern and Northeastern, it is part of the region's culture and is consumed in salty dishes, as a cream mixed with cassava or *tapioca* flour and also with fried fish (Portinho et al. 2012; De Arruda et al. 2014; Cialdella et al. 2014). That is totally different from what is found in the Southern and Southeastern regions were these fruits come as frozen pulp and are consumed as an ice cream / smoothy, beaten with *guarana* (*Paulliniacupana*) syrup and/or added of fruits (banana – *Musa x paradisiaca* or strawberry – *Fragaria x ananacia*) (Nascimento et al. 2008).

Due to its antioxidants (anthocyanins and flavonoids) vitamin C and fibers high content, is called a super fruit (Roges et al. 1996, 2000; Tonon et al. 2010; de Lima et al. 2015). Its pulp is also rich in lipids and is being highly consumed by the youngsters, sports people (as diet supplement) mainly as smoothie (frozen pulp-slushy/creamy drink) or juice (Menezes et al. 2008; Rufino et al. 2011, Portinho et al. 2012; Folly, 2014).

Despite of a rather lack of information regarding morphology characteristics of whole assai berry, some studies have been published by light microscopy (de Paula, 1975; Aguiar and de Mendonça, 2003; Tonon et al. 2009; Martins et al. 2009; Pessoa et al. 2010; Ribeiro, 2018). De Paula (1997), Aguiar and De Mendonça (2003), as well as Martins et al (2004), carried out light microscopy characterization of seed and the mesocarpicfibres / endocarpic. On the other hand, Tonon et al.. (2009) reported the assai morphological characterization of its powder product and Pessoa et al (2010) included scanning electron microscopy (SEM) of their fibers. Ribeiro followed the assai maturation structures and its correlation to moisture loss post-harvest (Ribeiro, 2018)

Considering that there is only a few information on assai (*whole fruit*) microscopy characteristics, the current study carried out its morphological characterization, through its different parts (skin / flesh / seed) differentiation by stereo microscopy (SM) and so their physical - dimensions/weight proportions.

II. Material And Methods

Material

Sample: fresh assai berries (500 g), at mature stage, from the main producing region of Northern Brazil (two sources: conventional and from extractivism i.e., producer I - PI and producer II – PII, respectively). Moisture content (mc) and water activity (a_w) of 46.3 and 48.5% & 0.9771 and 0.9851, respectively. Figure 1 presents the assai berry (*in natura*/fresh) from different origins.

Equipment: stereo microscope (10x and 0.71-11.5x for ocular and objective lenses respectively), Opzt, coupled to a color image-capture camera, model OPT14 MP, Opticam (Doral, Fl., USA); microbiological oven, Tecnal (Sao Paulo, SP, Brazil); a_w meter, Aqua- Lab4TE, Decagon (Sao Jose dos Campos, SP, Brazil).

Other materials: caliper, Digimatic (Tokyo, Japan); semi-analytical scale, PL300, Mettler (Barueri, SP, Brazil); drying oven, N515, Fanem (São Paulo, SP, Brazil); stainless stell scalpel, GS Mtronix (Sao Paulo, SP, Brazil).

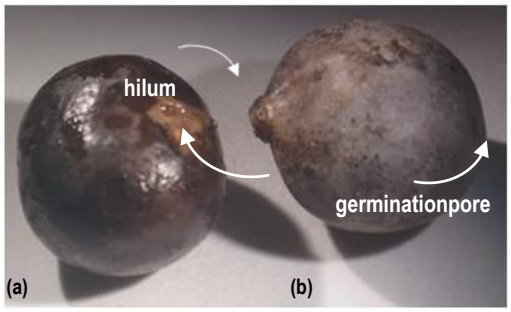


Figure 1. Assai (*Euterpeoleracea*Mart.) berries from two growing producers:
(a) PI - conventional and (b) PII - strativism.

Method

Assai collection and preparation:(a) collection - fruits, from both producers (PI & PII) were harvested directly from the assai trees by hand with gloves (at Belém city area, Pará state) and selected manually only the ones at mature stage (dark purple color skin). All were kept under refrigeration until the analyses were performed; *(b) preparation:* samples (n= 50 each) had their skin, flesh and seed (*whole and cross sectioned) separated for SM observation at different amplifications 1.7 to 50x (Scussel et al. 2014) (Figure 2).

Dimensions and weight: ***(a) dimensions*** - portions (50 units) of assai berries each origin (PI & PII), had the ***(a.1) whose fruits*** - measured for its height x width x depth (A x B x C) (Table 1); ***(a.2) proportions*** - their different parts also measured i.e., skin (its thickness), flesh (layer between skin and seed) and seed (diameter). That was carried out by means of a caliper. The hilum and the germination pore positioning were verified by measuring the distance between them (through height - both fruit sides and so width at fruit center); ***(b) weight*** - 50 units of both assai origins were weighed (whole) and their variables calculated (average, min., max., SD and RSD%) including each anatomical parts (total berry – 100%: skin x flesh (pulp/fiber) x seed %) and their proportion (Dominici, 2016).

Stereo microscopy: the assai berries tissues of each parts, either the skin (outer and inner surfaces), flesh (with pulp mesocarpic fibers) and seed (surface and cross sectioned with endocarpic fibers), as well as their botanical differentiations (hilum and germination pore), were observed by SM at different amplifications and registered (Scussel et al. 2014; Kreibich et al. 2016).

Humidity:(a) mc - was by gravimetry (AOAC, 2019), based on the weight loss of the material, with slow heating application in oven, until constant weight and *(b)* a_w- performed through the a_w meter device, at 25°C (Decagon, 2005).

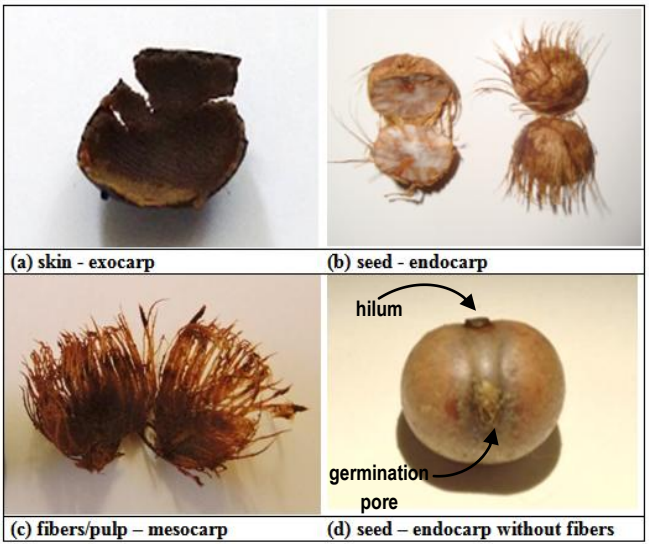


Figure 2. Assai (*Euterpeoleracea*Mart.) berries* parts: (a) skin, (b) seed in cross-section withendocarpic fibers, (c) mesocarpic fibers and (d) endocarp without fibers [1.7-10x].

III. Results And Discussion

From the assai fruits physical (dimensions, weight and each parts proportions *versus* whole) and stereo microscopy (skin, flesh and seed) data obtained, it was possible to establish their detailed characteristics. The physical (dimensions / weight / parts proportions) and morphological (different tissues structures) characteristics of the whole assai drupe are shown in Table 1 and Figures 3 to 5.

Physical characteristics

As far as the assai physical characteristics are concerned, data showed that they present a rather round to oblong shape with variation on fruit dimensions and parts proportions - depending on their origin. No detailed, dimensions, weight and proportions of assai berries parts (skin/flesh/seed) data have been reported in the literature to date. Table 1 presents the dimensions / shape and weights of assai berry (whole fruit *versus* its parts), including seed details and their variables.

Dimensions

(a) whole fruit - as expected, data showed differences among the fruits from different origins being the PII of larger size than PI. Their height, width and depth average for both assai North region sources (PI & PII) were of 11.8 mm (11.0-13.2); 13.6 mm (12.1-15.8) and 11.7 mm (11.4-13.8) for PI and 13.3 mm (12.3-15.8); 14.1 mm (12.3-16.8) and 12.3 mm (11.0-13.0) for PII. Those dimensions data confirm fruits size variation and the oblong (width higher than height (Figure 2).

(b) fruit parts proportions - the skin, flesh (pulp+fiber) and seed proportions (thickness: mm and %) obtained from the whole fruit were quite different, being most of the assai structure taken by the seed. While the***(b.1) skin***takes only 0.40 mm (0.31-0.72) and 0.66 mm (0.51-0.72) layer, which corresponds to 3.39 and 5.26% of total fruit for PI and PII, respectively, and the***(b.2) flesh (pulp & fibers)***, also a quite thin layer with 1.51 mm (1.12-1.61) and 1.62 mm (1.32-1.68) which corresponds to 12.79 $ (9.49-13.64) and 12.18% (9.93-12.63) of whole fruits, depending on the origin (conventional/cultivated or extractivist/forest). The***(b.3) seed*** - occupies most of the whole berry fruit (9.87-10.91 mm diameter for PI & PII, respectively). ***Fruit parts proportions:***therefore, the assai fruit dimensions (average) regarding skin (thickness), flesh (layer) and seed (diameter) of both origin (PI/PII) were of 0.40/0.66, 1.51/1.62 & 9.87/10.91 mm, respectively, corresponding to 3.39/4.96, 12.79/12.18 and 83.64/82.03%.

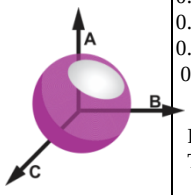
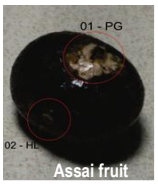
(c) seed parts - the assai endocarp data showed its round shape, despite of the whole fruit shape variation (oblong to roundish). The ***(c.1) coat*** - it is comprised of a thin tissue (0.15 to 0.3 mm; 1.44 to 2.89% - whole seed) covered with an average of 0.24 mm (0.15 to 0.31) and 0.23 mm (0.20 to 0.24) corresponding to 2.31% (1.44-2.98) and 2.15% (1.87-2.24) for PI and PI. The ***(c.2) endosperm*** – including the albumen, measures 10.16 mm (10.25-10.09) and 10.47 mm (10.46-10.50). i.e., 97.70 % (97.02-98.56). The ***(c.3) embryo*** – was not possible to visualize/measure it due to its small size and difficulty to distinguish/identify it by SM. Aguiar and Mendonça (2003) reported assai proportions, which were corroborated by our findings. Authors reported seed as

a globe-shape that occupies the most (60 to 75%) of the whole fruit. ***Seed parts proportions:***0.24 mm (coat) x 10.16 mm (endosperm) & 0.23 x 10.47 mm i.e., 2.31 x 97.7% & 2.15 x 97.87 % for PI & PII.

Weight

As expected, the whole assai berry weight in the current study varied with average 2.07 g (2.07-2.10) and 2.11 g (2.09-2.14), for the two fruit sources, respectively). Regarding the different assai parts weight and their proportions against the whole fruit (2.07 g - PI), they were of 0.17, 0.70 and 1.20 g, respectively for skin/pulp-fiber/seed coat and fruit from extractivism (2.11 g - PII) of 0.09, 0.52, and 1.50 g, respectively. Peso total 2.07 g (100%) – seed 1.20 g (57.97 to 73.2%; skin – 0.89 g (42.03 to 27%). The fruits weight reported by Pessoa et al (2010) was lower than the current work (1.46+/-0.26 g). No origin of the fruits was registered.

Table 1. Assai berry (*Euterpeoleracea* Mart.) physical characteristics from different origins

Assai samples[a]		Whole fruit DIMENSIONS (mm)			Humidity		Fruit & seed parts PROPORTIONS mm(%)				
							Fruit			Seed*	
Source/origin	Weight (g)	A (height)	B (width)	C (depth)	Mc[b] (%)	A_w[c]	Skin	Flesh	Seed	Ct[d]	En[e]+ Al[f]
Producer I[g]											
Conventional					46.3	0.9771					
Average	2.07	11.8	13.6	11.7			0.40(3.39)	1.51(12.79)	9.87(83.64)	0.24(2.31)	10.16(97.70)
Min	2.04	11.0	12.1	11.4			0.31(2.63)	1.12(9.49)	9.52(80.68)	0.15(1.44)	10.09(98.56)
Max	2.10	13.2	15.8	13.8			0.72(6.10)	1.61(13.64)	9.89(83.81)	0.31(2.98)	10.25(97.02)
SD±	0.322	0.759	1.188	0.888			0.128	0.190	0.132	0.149	0.350
RSD%	13.970	6.377	9.355	7.175			RSD%: 17.902 Total: 11.8 mm (100%)			RSD%: 0.931 Total: 10.4 mm (100%)	
Producer II[i]											
Extractivism					48.5	0.9852					
Average	2.11	13.3	14.1	12.3			0.66(4.96)	1.62(12.18)	10.91(82.03)	0.23(2.15)	10.47(97.76)
Min	2.09	12.3	12.3	11.0			0.51(3.84)	1.32(9.93)	9.10(68.42)	0.20(1.87)	10.46(98.13)
Max	2.14	15.8	16.8	13.0			0.72(5.41)	1.68(12.63)	11.21(84.21)	0.24(2.24)	10.50(97.85)
SD±	0.0176	0.9486	1.1005	0.6749			0.069	0.121	0.816	0.344	0.289
RSD%	0.003	0.921	1.211	0.534			RSD%: 21.981 Total: 13.3 mm (100%)			RSD%: 1.920 Total: 10.7 mm (100%)	

[a] 50 each [b] moisture content [c] water activity [i] from small property / forest [d] seed coat (thickness) [e] ruminateendosperm and [f] albumen (diameter) [g] plants cultivated in large scale [h] of the whole fruit * cross section =1/2 □

Morphological structures

The assai berry parts of the skin (surfaces: inner and outer), flesh (pulp, fibers - mucilaginous cell exudates) and seed (coat, endosperm-albumen) morphological characteristics were identified and shown in Figures 3 to 5. As well as fruit/seed two differentiation structures (germination pore and hilum).

(a) Skin (outer and inner surfaces): it is glabrous layer responsible for protecting berry inner content against mechanical actions and controlling the fruit moisture (Figure 3, 4 and 5.a). Its characteristic color (deep purple) is due to the anthocyanin pigments high content. From the assai ***(a.1)outer surface*** - it was possible to see the shiny and smooth surface of dark purple to bordeaux color, presenting some small lumps (stone cells) distributed throughout its area (Figures 3.a and 4.a). It has two morphological differentiation - *the germination pore* and *hilum*(Figures 2.d, 3a,c and 4a). The *germination pore* corresponds to the site where the seed bud will occur (shoot: stem & leave) and the *hilum* (region where the berries are attached to the tree - site that can get fungi spores contaminated. From the hilum different skin cell and fibrous tissues (extended up to the *germination pore*) (Figure 3.d). On the other hand, the skin ***(a.2)inner surface*** - is a structure that presents more well defined polygonal cells, with thick walls that get in contact to the flesh (cells / pulp exudate) (Figure 3.b and 4.b, respectively). It can be also seen some mesocarpic fibers scars close to hilum (Figure 5a) as well as lipid droplets (Figure 3.d).

(b) Flesh:it is located between skin and seed, in a rather thin layer of cells (of thin membranes) and full of nutrients (the pulp). It is surrounded by bundles of mesocarpic fibers and so endocarpic fibers (close to the seed coat) reserve cells, ***(b.1) pulp*** - although the assai is known for its semi-processed product - the pulp, it indeed, represents a quite small part of the whole berry (only 10 - 12% or less). It is seen as layers of hyaline mucilaginous reserve cells located right bellow the skin, next to the seed and distributed under and in between the thick bunch of fibers (Figure 1.c, 3.b.2 and b.4, 4.b and 5.b). ***(b.2) fibers*** – they are the second layer of resistance under the skin (in between flesh/pulp and surrounding the seed. They are also called, the mesocarp fibers. They are covered with pulp content (mucilage and lipids droplets) (Figure 5.b). Ribeiro (2018) reported that those fibers also get into the endocarp (seed), then called endocarp fibers (Figure 1.b). When assai pulp is extracted from the fruit, both reserve material and fibers come together (including skin) and its hydrosoluble pigments – the anthocyanins producing a thick juice "the assai wine" (anthocyanin, pulp and fibers rich) (Aguiar and Mendonca, 2013).

(c) Seed: the endocarp corresponds to a rather roundish and large structure (when related to the whole fruit diameter) that takes more than 80% of the total fruit and is covered with endocarp's fibers attached to its surface. It is very resistant, and under cross-section it is possible to see its structures – the seed coat (cell layers), the ruminate endosperm (brown) and its albumen (white), the accumulated food reserve (Figure 5c). Ruminate endosperm is a common condition in the palms. It is dissected by in-growths of the seed coat, i.e., it intrudes inwards via meristematic growth). Aguiar and de Mendonça (2003) describe the seed as a large, homogeneous, solid and hard endosperm with one germination pore, corroborating the current work observations.

IV. Conclusion

Regarding dimensions, the assai berries (both origin) widely varied from 11.0 to 15.8 mm (*height*) and 12.1 to 16.8 mm (*width*), with an oblong to roundish shape. The proportion between skin, flesh and seeds average was 82.0 to 83.6% (*seed*) and 16.2 to 17.2% (*skin + flesh* altogether). Their weight varied from 2.04 to 2.14 g despite origin (PI & PII).

Through SM, the *morphological* characteristics of different parts of assai Amazon palm berry were possible to identify. Some specifics such as the mesocarp – formed with numerous fibers surrounding the pulp cells, which represents a such small/proportion of the fruit (the main part for the assai food / pulp industry) in comparison to the rather large part (>80%) that represents the seed (a residue problem environment wise.

Next step, authors will evaluate the scanning electron microscopy berry histology, as most of the literature has been focusing on assai fruit composition. Also search for contaminations such as living organisms (insects, mites, fungi) that can reach - from inadequate handling/storage in the forests - is a need.

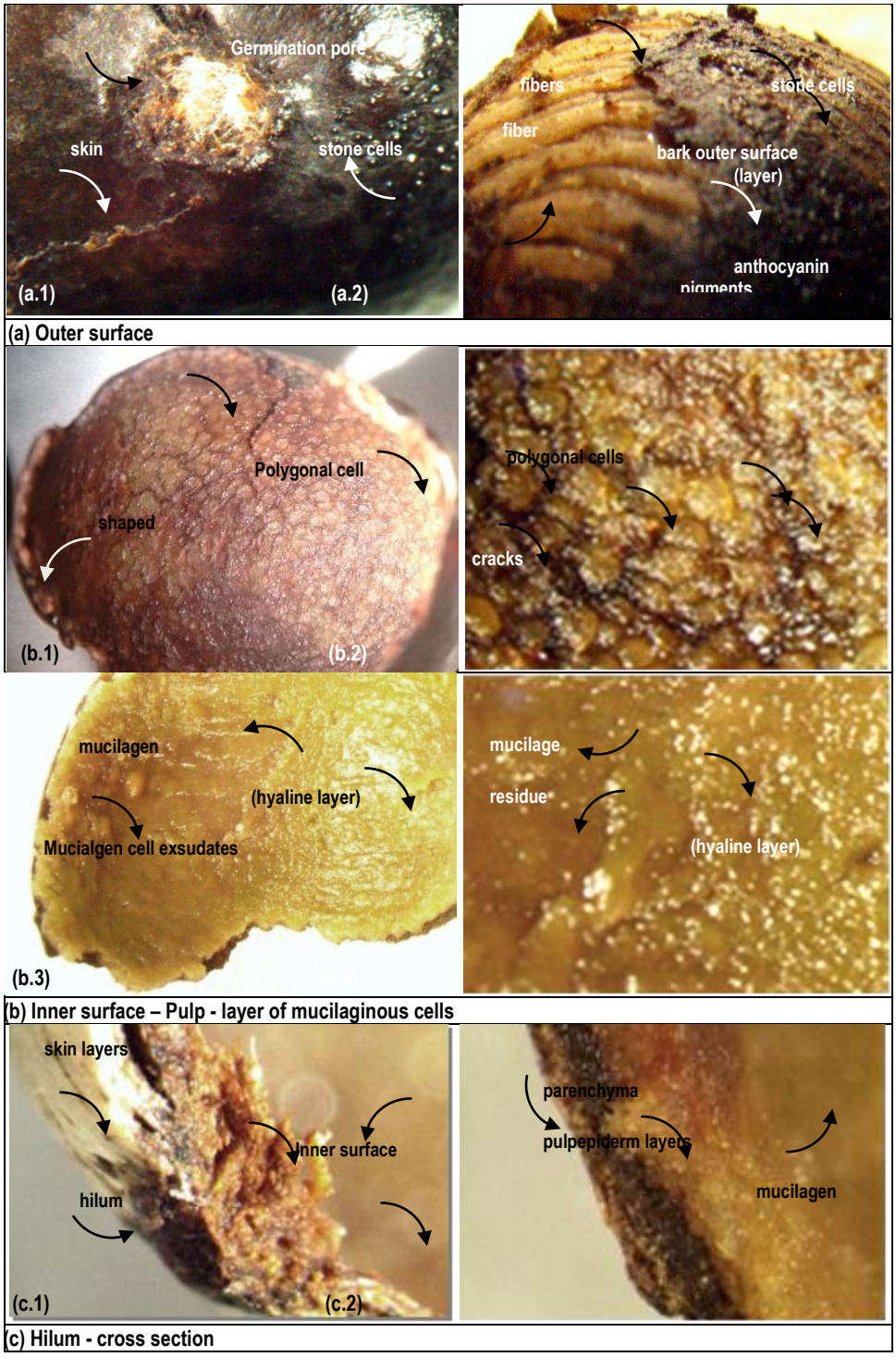


Figure 3.Stereo micrographs of assai (*Euterpeoleracea* Mart.)BERRY: (a) skin outer surface, (b) inner surface with pulp layer and (c) hilum - in cross section [1.7 to 20x].

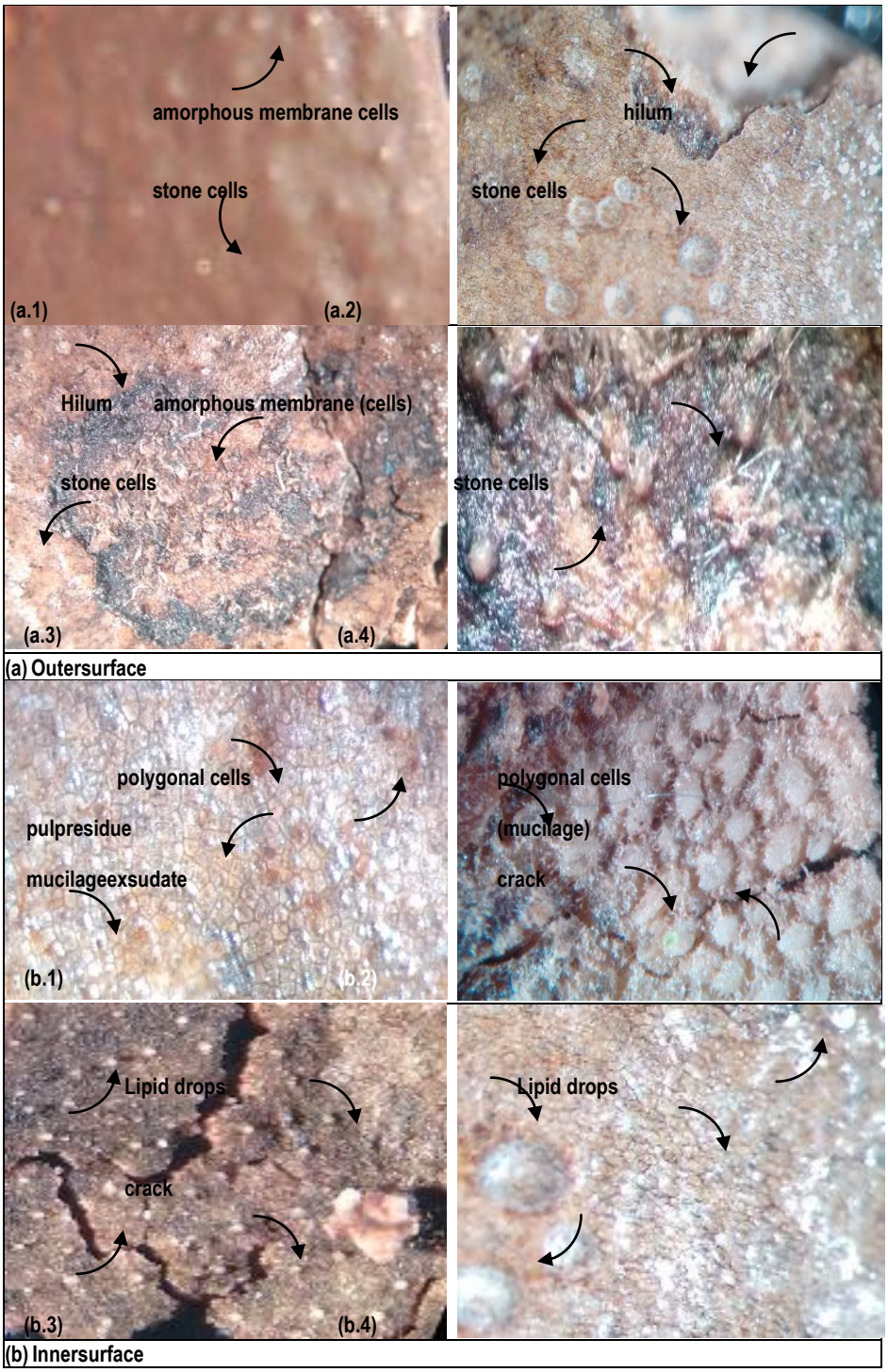


Figure 4. Stereo micrographs of the assai (*Euterpeoleracea* Mart.) berry SKIN: (a) outer and (b) inner surfaces [20 to 40x] dehydrated [20 to 40].

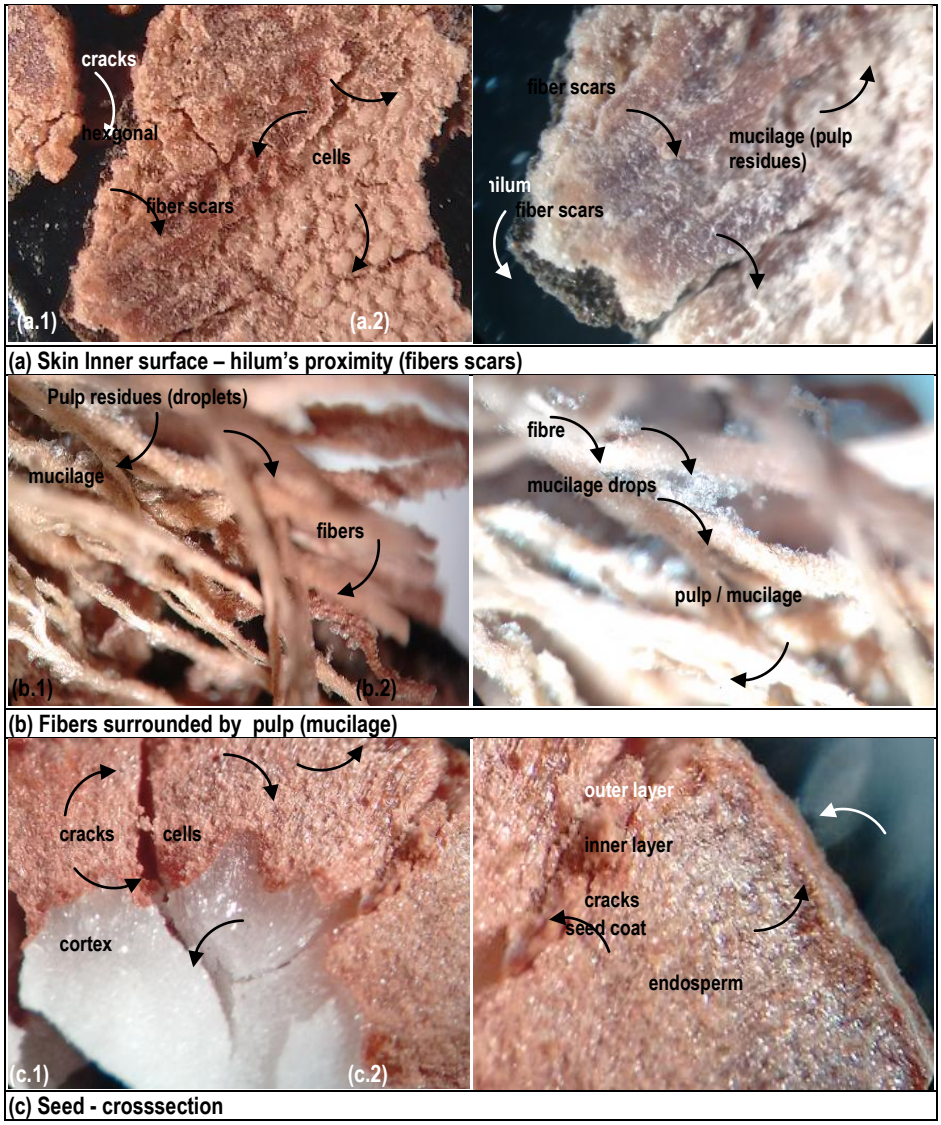


Figure 5. Stereo micrographs of the assai (*Euterpeoleracea* Mart.) berry SKIN/ PULP / SEED: (a) skin inner surface - hilum's proximity, (b) pulp region & fibers containing pulp residues and (c) seed cross sectioned [20 to 40x].

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