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Research Article

Developmental Variation in Physicochemical Properties and Selected Metabolites in *Alibertia edulis*, a Native Fruit from the Brazilian Cerrado

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Abstract

Alibertia edulis is a shrubby fruit species native to the Brazilian Cerrado biome, with recognized nutritional and medicinal potential. Commonly known as marmelada-de-cachorro, marmelinho-do-Cerrado, marmelada-do-campo, or puruí-miúdo, it produces berry-like fruits with dark purple pulp, consumed fresh or processed by local populations. This study aimed to characterize the physicochemical and biochemical changes in *A. edulis* fruits during development. Fruits were collected every 10 days from anthesis to full ripening in an experimental orchard of native Cerrado species. Throughout the 72-day developmental cycle, there was a marked increase in fruit mass (4.6 to 13.5g), soluble solids (7.75 to 19.42 °Brix), and total sugars (1.6 to 14.33g 100 g^{-1}), while titratable acidity declined from 2.1 to 0.6 g citric acid 100 g^{-1}. The peak starch content (17.92g 100 g^{-1}) at 60 DAA was followed by its conversion into soluble sugars, indicating the onset of ripening. The dark purple coloration observed after 70 DAA coincided with chlorophyll degradation and the appearance of anthocyanins, with levels reaching 4.78 mg 100 g^{-1} in both peel and pulp at 72 DAA. Carotenoids, initially present in the pulp, decreased sharply during this period, likely replaced or masked by anthocyanins. Organic acids such as tartaric, malic, lactic, acetic, oxalic, and formic were identified, with tartaric acid showing a 317% reduction (from 0.13 to 0.041g 100 g^{-1}), supporting the reduction in overall acidity and rise in pH (up to 5.62). These transformations reflect intense metabolic activity associated with fruit ripening, suggesting that *A. edulis* is a valuable source of functional compounds, including anthocyanins, carotenoids, and organic acids. The combination of high sugar content, moderate acidity, and bioactive compounds enhances the fruit's appeal for both fresh consumption and industrial processing, reinforcing its potential as a functional food and the importance of conserving Cerrado biodiversity.

Keywords: Brazilian Cerrado; Ripening Physiology; Organic Acids; Carotenoids; Postharvest Potential

Abbreviations

DAA: Days After Anthesis; SS: Soluble Solids; TA: Titratable Acidity; FW: Fresh Weight; DW: Dry Weight; Ph: Potential of Hydrogen; g 100g^{-1}: Grams per 100 grams of fresh weight.

Introduction

Alibertia sessilis Schum., popularly known as "marmelada-de-cachorro," "marmelinho," or "marmelo-do-cerrado", belongs to the Rubiaceae family, a native fruit tree species of the Cerrado ecoregion in Brazil's Cerrado. It thrives across several states [1,2]. It is a fruit and medicinal species of great importance in the Cerrado biome. Its wood is used for firewood and charcoal; the leaves are consumed by cattle and, along with the branches, are traditionally used in the form of poultices, compresses, or medicinal baths for the treatment of skin disorders [3,4]. The fruits, in addition to being edible, are highly appreciated by regional bird species. They ripen between November and February and present a berry-like shape, ranging from 1.5 to 3.0 cm in diameter, with a blackish-violet epi-

carp, fleshy mesocarp, and dark greenish-brown pulp containing numerous seeds [4,5].

There are many challenges associated with the exploitation of native fruits; however, they also present a significant potential, especially regarding their sustainable use and export value, given their unique flavors and the fact that they are not found in other countries [3]. Studies focusing on the development and ripening physiology of Cerrado species are essential to generate information that supports both conservation in natural habitats and the establishment of commercial cultivation systems, helping to prevent predatory extraction. Moreover, such knowledge contributes to defining appropriate harvest times and applying postharvest technologies that reduce losses and improve fruit utilization [1,6,7].

Nevertheless, research on the developmental stages of Cerrado native species is still incipient, and little is known about the distinct phases of fruit growth, pre-ripening, ripening, maturation, and senescence [8]. Therefore, the objective of this study was to evaluate the physical and chemical changes during the development of marmelada-de-cachorro fruits, aiming to determine the most suitable maturity stage for harvest and to support the adoption of appropriate production and preservation techniques.

Materials and Methods

Experimental site and plant material

The study was conducted in an experimental orchard composed of eleven native fruit species from the Cerrado biome, located at the School of Agronomy of the Federal University of Goiás (EA/UFG), Goiânia, GO, Brazil (16°35'12" S; 49°21'14" W; 730 m altitude), on a dark red Latosol. Meteorological data were obtained from the EA/UFG Evaporation Station. Fifteen *Alibertia sessilis Schum*. trees that produced flower buds were selected from a total of 24 individuals. Flowers were tagged at anthesis using colored wool threads. The first fruit collection was performed at 10 days after anthesis (DAA), with subsequent samplings every 10 days up to 72 DAA, when fruits reached full maturity, indicated by their blackish-violet peel color, totaling eight sampling points.

Sampling and preparation

Fruits were harvested randomly in the morning, packed in polyethylene bags, and transported to the laboratory. A total of 800 fruits were collected at 10 DAA, 400 fruits at 20 and 30 DAA, and 200 fruits at each of the later stages. Fruits were divided into four equal batches, representing replicates. Immediately after harvest, firmness (N) was measured using a Stable Micro Systems TA.XT2i texture analyzer with a 2 mm needle probe (P/2N), operating at 5 mm s^{-1} speed and 5 mm penetration depth. Peel color was measured at three equidistant points using a Hunter Lab ColorQuest XE colorimeter in CIE Lab* mode. Fruit mass (g) was measured on a semi-analytical balance, and longitudinal and transverse diameters were recorded using a digital caliper. Relative growth rates for mass (g day^{-1}) and diameters (mm day^{-1}) were calculated using the formula:

$$RGR = (V1-V0)/ (T1-T0)$$

Where RGR = relative growth rate, V = variable value (initial and final), and T = time (DAA). Fruits were then frozen in liquid nitrogen and stored at −18 °C for subsequent analyses.

Physicochemical analyses

The pH was measured using a Schott Handylab pH meter [9]. Titratable acidity (TA) was determined by titration with 0.1 N NaOH, using phenolphthalein as an indicator, and expressed as % tartaric acid [9]. Soluble solids (SS) were measured using an ATAGO PR-100 digital refractometer with automatic temperature compensation at 25 °C and expressed in °Brix [9]. The SS/TA ratio was calculated by dividing SS by TA.

Sugar, starch, and pectin content

Total soluble sugars (% glucose) were extracted with 70% ethanol and quantified spectrophotometrically at 620 nm using the anthrone method, with results expressed in g 100 g^{-1} [10]. Starch was extracted and hydrolyzed chemically, then quantified using the Somogyi–Nelson method [11] and expressed in g 100 g^{-1}. Soluble and total pectin were extracted according to McCready and McColomb [12] and quantified at 520 nm using the Blumenkrantz and Asboe-Hansen [13] method, with results expressed as mg galacturonic acid 100 g^{-1}.

Organic acids

Organic acids (tartaric, malic, lactic, acetic, oxalic, and ascorbic) were quantified by high-performance liquid chromatography (HPLC) using an HP 1100 system (Agilent) equipped with a quater-

nary pump, degasser, automatic injector (20 µL), and diode array detector (DAD). Detection was set to 250 nm for ascorbic acid and 210 nm for the remaining acids.

Pigment analysis

Total chlorophyll content was determined from 1 g of fruit peel homogenized in 10 mL of water and brought to 50 mL with acetone. After dark rest and filtration, absorbance was read at 652 nm, and chlorophyll content was calculated using Engel and Poggiani [14], expressed in mg 100 g^{-1}. Total carotenoids were extracted from 10 g of pulp using an isopropanol:hexane (3:1) solution, washed, filtered with sodium sulfate, and brought to 50 mL with acetone and hexane. Absorbance was read at 450 nm and results expressed in mg 100 g^{-1} [15]. Total anthocyanins were determined by extracting 0.5 g of sample with a 95% ethanol + 1.5 N HCl solution (85:15), followed by cold storage, filtration, and spectrophotometric reading at 535 nm. Results were expressed as mg 100 g^{-1} [16].

Experimental design and statistical analysis

The experiment was conducted in a completely randomized design with eight collection periods and four replicates. Statistical analyses were performed using RStudio. Principal Component Analysis (PCA) was used to reduce data dimensionality and identify associations among the stages of fruit development and the evaluated variables. Before PCA, all variables were standardized (z-score), and the first two principal components were used to construct biplots representing both the contribution of variables and the distribution of the sampling points in the multivariate space.

Results and Discussion

Flowering, fruit set, and growth pattern

In Goiânia, Brazil, the flowering of *Alibertia sessilis* was first observed in late August, reaching its peak in September. Initial fruit set occurred at the end of September, peaking in October, and December was identified as the ideal harvesting period. The entire cycle from flowering to harvest spanned 72 days. The fruit developmental stage was considered from flower opening (anthesis) to harvest, which was defined by the ease of fruit detachment from the shrubs and the appearance of a blackish-violet skin coloration. During the study period, air temperature ranged from 21.3 to 26.4 °C, relative humidity varied between 57% and 80%, rainfall ranged from 0.0 to 262.6 mm, and total solar radiation ranged from 136.3 to 275.6 hours. November stood out due to well-distributed rainfall, with precipitation recorded on 19 days during the month (EE/EA).

The development of seeded fruits begins with fertilization and progresses through stages such as fruit set, growth, maturation, ripening, and senescence [17,18]. Anthesis can be considered the starting point of fruit development, whether in parthenocarpic or seeded species. The duration from anthesis to ripening varies across fruit species [8,19,20].

Throughout the 72-day development period, there was a significant increase in longitudinal diameter (LD), transverse diameter (TD), and fruit mass (M) of *A. sessilis* ($p < 0.05$) (Figure 1A and 1B), following a simple sigmoidal growth pattern over time. This type of growth is commonly observed in fruits such as apples, bananas, and oranges, while double sigmoid patterns are found in peach, cherry, and grape [21,22]. Maximum values for these variables were recorded at 72 DAA: fruit mass of 25.91 g, LD of 34.67 mm, and TD of 31.92 mm, coinciding with the stage in which the fruit exhibited full blackish-violet skin coloration. Similar diameter values in mature fruits of *A. sessilis* were reported by Matheus., *et al.* [5], who described average diameters of approximately 33 ± 2 mm. The relative growth rate (RGR) of longitudinal and transverse diameters showed similar trends, with peak growth rates at 20 DAA (1.01 mm day^{-1} and 1.05 mm day^{-1}, respectively), followed by a gradual decline (Figure 1C). For fruit mass, the RGR increased steadily until 60 DAA, reaching a maximum of 0.130 g day^{-1}, and then decreased slightly until 72 DAA (Figure 1D).

Physicochemical Ripening Indicators during Fruit Development

During the development of Alibertia sessilis, significant changes were observed in key physicochemical quality parameters (Figure 2A–D). Soluble solids (SS) showed a substantial increase after 60 DAA, reaching a maximum of 22.8% at 72 DAA. The data followed a cubic regression model with a coefficient of determination of R^2 = 94.73%, reflecting a consistent accumulation of sugars, which is typical of the ripening process in climacteric fruits, where starch

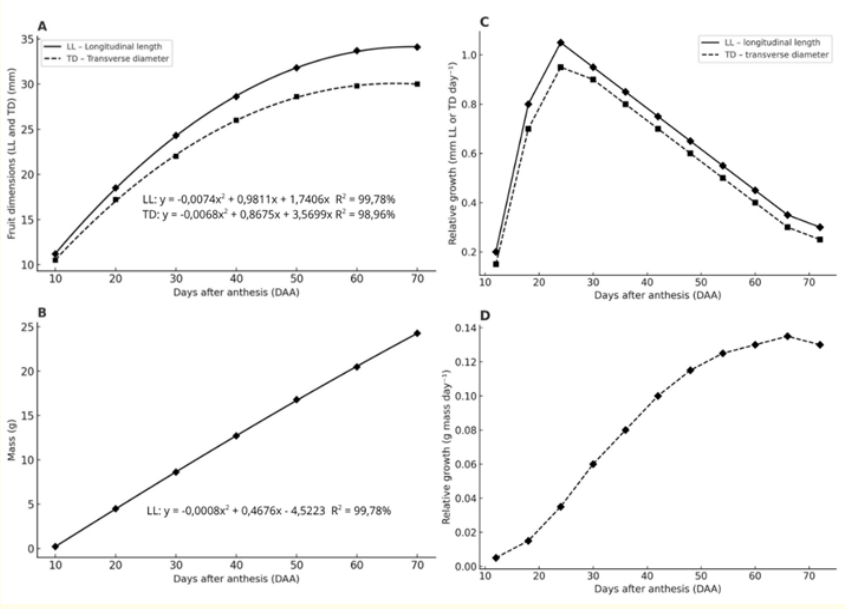

Figure 1: Mean longitudinal length and transverse diameter (A), fresh mass (B), and their respective relative growth rates (C, D) of *Alibertia sessilis* fruits from 10 to 70 days after anthesis (DAA).

reserves are hydrolyzed into simple sugars, intensifying the sweet flavor characteristic of mature fruit [23].

Titratable acidity (TA) declined steadily throughout the developmental period, from 3.76% to 1.80% citric acid, and was best described by a quadratic regression model with R^2 = 91.85% (Figure 2B). This pattern mirrors findings from other studies on fruit development in Cerrado species [7,8]. This decrease is often associated with the metabolic consumption of organic acids during respiration and their conversion into sugars, which is also supported by the increase in pH values during ripening [24].

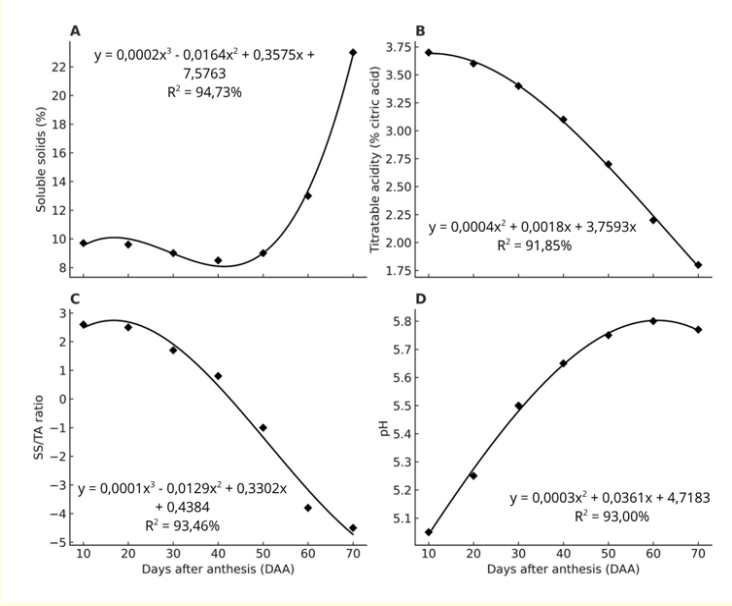

Figure 2: Mean values of (A) soluble solids, (B) titratable acidity (as tartaric acid), (C) SS/TA ratio, and (D) pH in Alibertia sessilis fruits during development.

The SS/TA ratio increased significantly from 60 DAA, peaking at 12.94 at full maturity (72 DAA), following a regression model with R^2 = 93.46% (Figure 2C). This ratio is a critical parameter for flavor quality, reflecting the balance between sweetness and acidity, and is widely used as a ripeness index for harvest and processing decisions [25,26].

The pH values showed a gradual increase over time, reaching a peak of 5.81 at 70 DAA, with a strong regression fit (R^2 = 93.00%) (Figure 2D). The rise in pH reflects the breakdown of acidic components and supports the transition to fruit maturity [24,26]. These combined changes in SS, TA, the SS/TA ratio, and pH confirm the physiological progression toward optimal fruit quality, highlighting the potential of A. sessilis for both consumption and industrial applications.

According to da Silva., *et al.* [26], the interaction between acidity and soluble solids is a key determinant of fruit acceptance, as lower acidity enhances the perception of sweetness. Furthermore, the increase in soluble solids during ripening is indicative of polysaccharide degradation and their conversion into soluble sugars, contributing to a more favorable sensory profile.

Carbohydrate metabolism and organic acid dynamics during fruit development

Starch, total soluble sugars, and organic acids exhibited distinct and complementary behaviors during the development of Alibertia sessilis fruits (Figure 3A–C). Starch content increased steadily from 10 to 60 DAA, reaching a maximum of 17.92 g.100g^{-1}, followed by a noticeable decline by 72 DAA, indicating active hydrolysis and conversion into sugars during ripening (Figure 3A). This transition coincided with a sharp increase in total sugars, which rose from 1.41 g.100g^{-1} at 10 DAA to 12.81 g.100g^{-1} at 72 DAA (Figure 3B). The curve fit for sugar accumulation showed a strong determination coefficient (R^2 = 98.9%), reflecting the progressive and consistent sugar buildup in the pulp.

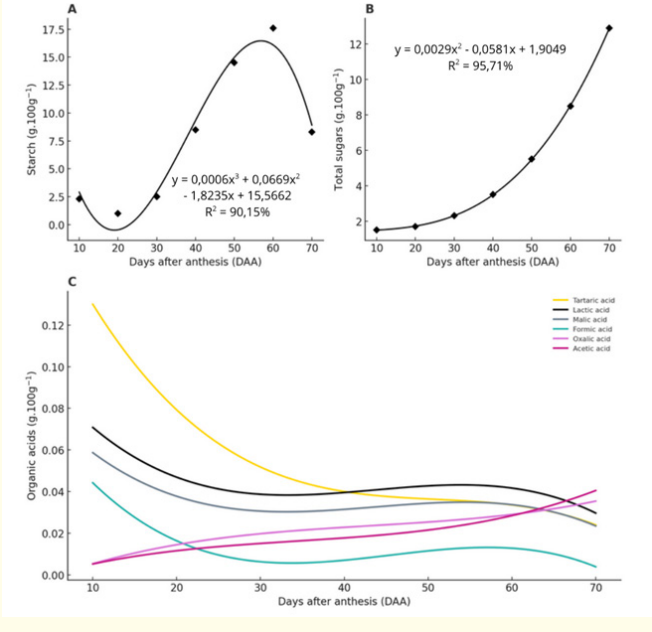

Figure 3: Mean values of (A) starch, (B) total soluble sugars, and (C) organic acid profile (formic, lactic, acetic, tartaric, oxalic, and malic acids) in Alibertia sessilis fruits during development.

This starch-to-sugar shift represents a classic metabolic pattern of climacteric-like fruit development, where energy reserves stored during early growth are mobilized into soluble sugars, enhancing sweetness and contributing to organoleptic quality. These metabolic changes are in line with the increase in soluble solids and SS/TA ratio discussed previously [7,27,28].

In parallel, the organic acid profile underwent significant transformations (Figure 3C). Tartaric acid, initially the most abundant (0.102 g.100g^{-1} at 10 DAA), showed a consistent decrease throughout development, reaching 0.041 g.100g^{-1} at 72 DAA. Other acids, such as formic, lactic, and acetic acid, maintained relatively stable or slightly fluctuating concentrations, with malic and oxalic acids contributing modestly to the overall acidity. The general trend reveals a dilution or catabolic reduction of organic acids during ripening, which correlates with the decreasing titratable acidity and increasing pH observed in Figure 2.

These results highlight the biochemical foundation of flavor modulation in A. sessilis, where the interplay between sugar accumulation and acid degradation defines the sweetness-acidity balance critical for consumer acceptance and processing potential.

Skin color and pigment changes during ripening

Significant changes ($p < 0.05$) were observed in the skin color of Alibertia sessilis fruits during development, as indicated by the L*, a*, and b* color parameters. These variables followed a cubic trend: L* values increased up to 60 DAA and then declined sharply by 72 DAA; a* values showed a gradual increase, while b* values decreased steadily (Figure 4A, B, and C). Fruits initially exhibited green and yellow tones (a* = –14.80 and b* = 37.56), transitioning to a nearly neutral color by 72 DAA (a* = 0.32 and b* = –1.77). The relationship between L* and a* reflects the loss of green intensity, shifting from dark green (L* = 23.33; a* = –14.80) at 10 DAA to light green (L* = 56.09; a* = –5.58) at 60 DAA.

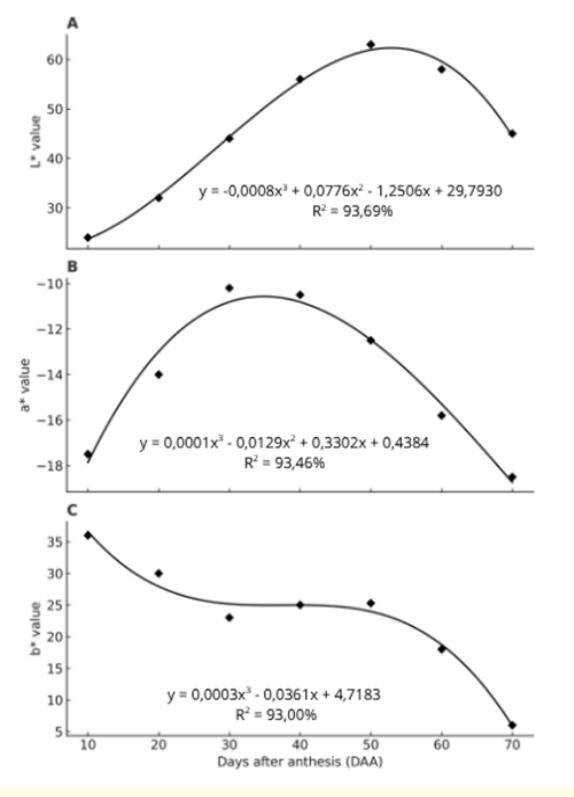

Figure 4: Mean values of the color parameters L* (a), a* (b), and b* (c) of Alibertia sessilis fruits during development.

This pattern was accompanied by a significant ($p < 0.05$) reduction in total chlorophyll content (Figure 5A), likely due to pigment degradation mechanisms such as structural disassembly, pH variation caused by the accumulation of organic acids in vacuoles, and the activity of oxidative systems and chlorophyllases [21,24]. The onset of blackish-violet coloration was marked by a decline in L* and b* values and a progressive increase in a*, particularly around 70 DAA (L* = 18.57, a* = –3.76, b* = 7.66), culminating in a completely dark-purple skin by 72 DAA (L* = 18.57, a* = 0.32, b* = –1.77).

The reduction in b* values was also associated with the degradation of carotenoids in the pulp (Figure 5B), which declined progressively throughout ripening and were nearly absent after 70 DAA, coinciding with the emergence of anthocyanin pigments. Total anthocyanins were detected at 70 and 72 DAA in the fruit skin (1.29 and 4.78 mg 100 g^{-1}, respectively), suggesting that skin color change is the result of both chlorophyll breakdown and anthocyanin synthesis. The anthocyanin levels detected were relatively low and associated with a* values between 0 and 1, indicating a transition from a neutral to reddish color tone.

During fruit development, the dynamics of pigment content in Alibertia sessilis revealed distinct biochemical transitions, particularly in chlorophyll, carotenoids, and anthocyanins. A marked decrease in total chlorophyll was observed from early stages (15.6 mg 100 g^{-1} at 10 DAA) to the final phase of maturation, reaching values close to 1.3 mg 100 g^{-1} at 70 DAA. This progressive degradation of chlorophyll is a typical ripening marker in climacteric and non-climacteric fruits and is associated with the loss of green coloration as the fruit approaches maturity (Figure 5A). The reduction in chlorophyll likely results from increased chlorophyllase activity and the activation of senescence-related genes, leading to the dismantling of chloroplast structures and the loss of photosynthetic capacity [21,24,29].

Concurrently, total carotenoids also decreased, from approximately 4.0 to 0.7 µg 100 g^{-1} over the same period. Although carotenoids generally accumulate during ripening in many fruit species, their decline in A. sessilis suggests that these compounds may primarily serve photoprotective roles during early fruit development, being gradually replaced or degraded as ripening progresses. Similar trends have been reported in other native Cerrado species, where the initial carotenoid pool may not significantly contribute to pigmentation but rather to physiological stability during growth [30,31].

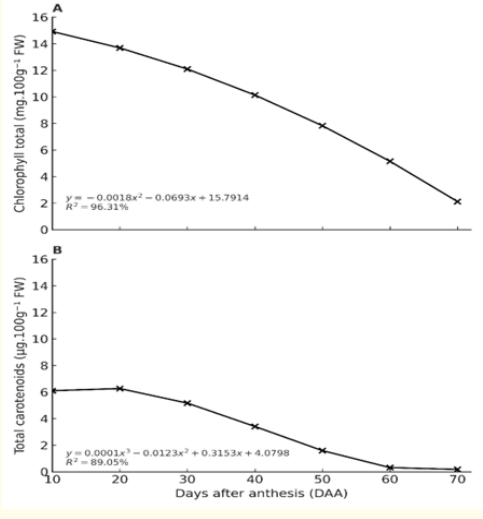

Figure 5: Mean values of total chlorophyll (A) and total carotenoids (B) in Alibertia sessilis fruits during development.

Interestingly, the reduction in chlorophyll and carotenoids preceded the visible appearance and accumulation of anthocyanins in the pulp (Figure 5B). Anthocyanin concentrations increased sharply at the final developmental stages, reaching 3.67 and 4.78 mg 100 g^{-1} at 70 and 72 DAA, respectively. These values are in agreement with those documented by Rocha [32], in fully ripened fruits of A. sessilis from the Piauí Cerrado (4.30 ± 0.12 mg 100 g^{-1}). The transition from chlorophyll/carotenoid dominance to anthocyanin accumulation illustrates a typical pigment shift associated with ripening, where chloroplasts are converted into chromoplasts or other plastid types, and biosynthetic pathways of phenylpropanoids become increasingly active.

As Fischer and Bennet [33] describe, fruit color change during ripening is a multifaceted process involving the degradation of chlorophylls, the modulation of carotenoid levels, and the biosynthesis of flavonoid pigments such as anthocyanins. In *A. sessilis*, this sequence likely reflects a shift in metabolic priorities, from development and photoprotection to signaling readiness for seed dispersal. The visual cues resulting from anthocyanin accumulation may act as attractants for frugivores, while the degradation of chlorophylls and carotenoids marks the transition from growth to senescence [24]. Overall, the pigment profile of A. sessilis pulp not only defines its visual quality but also reflects underlying physiological and ecological strategies during fruit.

Multivariate relationships between morphological, biochemical, and pigment variables during fruit development

Principal component analysis (PCA) (Figure 6) explained 99.3% of the total data variance, with PC1 (87.9%) and PC2 (11.4%) effectively distinguishing the developmental trajectory of Alibertia sessilis fruits. PC1 captured the major biochemical and morphological transitions, clearly separating early stages (10–30 DAA), characterized by high levels of chlorophyll and titratable acidity, from advanced maturation stages (60–70 DAA), marked by an accumulation of sugars, carotenoids, anthocyanins, and increased fruit mass and pH.

The direction and strength of the variable vectors in the biplot revealed distinct metabolic patterns. Sugars, carotenoids, anthocyanins, soluble solids, and fruit mass clustered in the same quadrant of PC1, indicating their synchronized increase during ripening and their potential use as reliable indicators of ripeness. Conversely, chlorophyll and titratable acidity projected negatively on PC1, reflecting the degradation of pigments and decline in acid typical of senescence processes. The intermediate stages (40–50 DAA) were centrally positioned, indicating transitional dynamics between growth and ripening metabolism.

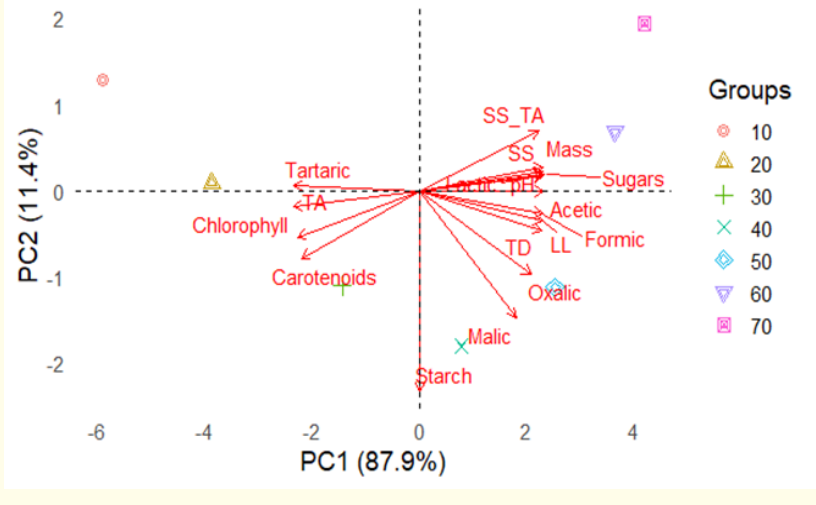

Figure 6: MPrincipal component analysis (PCA) of morphological, biochemical, and pigment-related variables during Alibertia sessilis fruit development (10-70 DAA).

This multivariate approach underscores how tightly coordinated the physical, chemical, and pigment-related changes are during the fruit's ontogeny. The overlapping vectors of sugars and anthocyanins with carotenoids reinforce their interdependence and their combined role in defining the visual and nutritional maturity of the fruit.

Taken together, these findings not only confirm the multistage physiological shift during A. sessilis fruit development but also suggest promising biomarkers, such as total soluble solids, carotenoids, and anthocyanins, for objective ripeness assessment. Future studies could explore predictive models based on these key variables or expand the analysis to other Cerrado native species for comparative developmental profiling. Additionally, integrating transcriptomic or metabolomic data could deepen our understanding of the regulatory networks that govern pigment biosynthesis and sugar accumulation in this underutilized tropical fruit.

Conclusion

The development of *Alibertia sessilis* fruits in the Brazilian Cerrado spans 72 days and follows a simple sigmoidal growth pattern, marked by increases in fruit mass, soluble solids, total sugars, pH, and soluble pectin, along with the degradation of starch, organic acids, chlorophyll, and total carotenoids. Maturation-related physiological and biochemical changes become more evident after 60 days after anthesis (DAA), culminating in the appearance of a dark purple (atropurpureous) skin, which indicates the optimal harvest stage.

Acknowledgements

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Conflict of Interest

The authors declare no conflict of interest.

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