Sensory and Physical Changes of Green Cubiu Fruits (Solanum sessiliflorum Dunal, Solanaceae) During the Post-Harvest Period at Ambient Atmosphere

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Abstract
Cubiu (Solanum sessiliflorum Dunal, Solanaceae) is an attractive fruit due, in part, to its exuberant appearance (e.g., great freshness, vivid colors, and large size). Besides, cubiu fruits are highly valued for their nutritional richness (e.g., soluble fibers such as pectin, vitamin C, potassium, and carotenoids). The objective of this study was to evaluate sensory and physical changes of green cubiu fruits during the post-harvest period at ambient atmosphere. Green cubiu fruits were randomly harvested and immediately taken to the laboratory of the Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus, Amazonas, Brazil. The perfect fruits were then selected (n = 20), stored at ambient atmosphere, and daily evaluated for (i) loss of weight, (ii) longitudinal (L) and transverse (T) diameter modifications, (iii) loss of freshness, and (iv) color changes. The duration of the experiment was defined by the total loss of freshness of all investigated fruits; thus, making their appearance potentially unfit for human consumption. The predominant shape of cubiu fruits was cordiform (1.17) throughout the experiment, i.e., yet suitable to easily peel. The loss of freshness started on the sixth post-harvest day at ambient atmosphere, being apparent in 100% of the fruits 17 days later (end of the experiment). Of importance, there was a statistically significant negative correlation between the temperature and the L/T ratio. The color changes started on the seventh post-harvest day at ambient atmosphere, but they were characteristically irregular, imperfect, and incompatible with three standard ripening stages of the fruit at the end of the experiment (i.e., eight fruits (40%) remained green, seven fruits (35%) reached the turning stage, and five fruits (25%) reached the ripe stage). No fruit reached the fully ripe stage. Harvested at the green stage, cubiu fruits remained attractive to consumers for five days at ambient atmosphere.

Keywords: Cocona; Color; Freshness; Shape; Shelf life; Weight.

1. Introduction
Brazil holds about 20% of the world's biodiversity [1]. The Central Amazonia is greatly representative of this 1/5 of the planetary biodiversity [2]. It is part of the largest remaining tropical forest in South America, being composed of the states of Amapá and Pará, the east of the state of Amazonas, and part of the state of Rondônia [2] [3]. Cubiu shrubs (Solanum sessiliflorum Dunal, Solanaceae) are not only found in this Brazilian region rich in flora and fauna, but also in other surrounding countries (e.g., Colombia, Peru, Bolivia, and Venezuela) [4].

Cubiu shrubs (Figure-1) produce fruits in the form of fleshy berries that are very attractive at any ripening stage (including the green one) due to their exuberant appearance (e.g., great freshness, vivid colors, and large size), in addition to their pleasant acidic taste, high content in carotenoids, vitamin C, and fibers (especially the water-soluble ones such as pectin) [4] [5] [6]. Furthermore, cubiu fruits are nutritionally dense in all their tissue portions (i.e., peel or exocarp, pulp or mesocarp, and placenta or endocarp) throughout the ripening process, especially in the placenta at the fully ripe stage [4] [5].

Interestingly, in the Amazonian region, fleshy berries such as cubiu are picked regardless of the ripening stage [5] [6]. This timing of the fruit harvest is used in part because the ingredients necessary to prepare the traditional fish stew are not easily available throughout the vast Amazonian territory; thus, the native population uses whatever foods are available to add substance to the daily meal [5] [6].

The period during which a food product remains acceptable and meets the consumers' expectations defines shelf life [7]. Storage temperature is one of the most important factors affecting the shelf life of fruits [8]. After harvest, cubiu fruits are usually kept at ambient atmosphere until consumption. In addition, freshness is the quality that indicates the vigorous character of a fruit (i.e., the fruit possesses the appearance of freshly harvested) [7].

Nevertheless, most fruits present colors as the central aspects of their identity, facilitating their detection and visual recognition [9]. Colors are also indicators of the ripening stages of fruits [7] [10]. Ripening marks the completion of development of a fruit and the commencement of senescence (an irreversible event) [11]. During

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ripening, cubiu fruits acquire vivid and distinctive colors due to the tissue predominance of different pigments (i.e., chlorophylls, flavonoids, and carotenoids) [4] [5]. Four colors are described as patterns for the recognition of the ripening stages of cubiu fruits, i.e., entirely green fruits; turning fruits (with intermediate colors between green and yellow); ripe fruits (with entirely yellow color); fully ripe fruits (dark red or wine) [10]. Of note, when cubiu fruits are at a very advanced ripening stage, they open spontaneously (dehiscence), becoming unfit for human consumption, but otherwise attractive for feeding bats and birds that enable seed dispersal [12].

Lastly, both the shape and the weight of fruits (in general) are genetically determined traits [4] [13]. Nonetheless, these traits are influenced by several factors (Figure-2), including those related to the post-harvest period, further discussed in the present study [4] [11]. According to previous works, these commercially important polygenic traits are variable in cubiu fruits [4][10][14].

Therefore, the objective of this study was to evaluate sensory changes (i.e., loss of freshness and color modifications) as well as physical changes (i.e., modification of shape and loss of weight) of green cubiu fruits during the post-harvest period at ambient atmosphere.

2. Materials and Methods

2.1. Sample Preparation

Green cubiu fruits (Solanum sessiliforum Dunal, Solanaceae) were randomly and manually harvested from plants grown at the Experimental Station of Olericulture Dr. Alejo Von Der Pahlen of the Instituto Nacional de Pesquisas da Amazônia (INPA), located in the surroundings of the municipality of Manaus, Amazonas (AM), Brazil (Km 14 of the state highway AM-010).

Immediately after harvest, the fruits were transported in plastic containers to the laboratory (INPA) where they were washed and dried at ambient atmosphere. Those fruits without any injury were selected for the study, composing a sample of 20 perfect green cubiu fruits. Each fruit was weighed (mean initial of 148.75 g) and carefully packed in a plastic tray (2 × 26 × 42 cm) covered with a double sheet of paper towel. The 20 fruits were then stored at ambient atmosphere (mean temperature of 29.24°C) for the present study.

2.2. Sensory and Physical Evaluations

The fruits were daily evaluated for (i) loss of weight (Filizola® semi-analytical balance, São Paulo, Brazil), (ii) modification of longitudinal diameter and transverse diameter (Starret® pachymeter, 0.001, California, USA), (iii) color changes and (iv) loss of freshness with image registration (FD Mavica digital camera, Sony, New York, USA).

The duration of the study was defined by the total loss of freshness of all fruits; thus, making their appearance potentially unfit for human consumption.

At the end of the study, the internal integrity of the fruits’ tissues (i.e., peel, pulp, and placenta) was also evaluated by means of a cross-section cut with stainless steel knife and image registration.

2.3. Statistical Analyses

This experiment was conducted in a randomized design comprising 20 perfect green cubiu fruits freshly harvested. The time (post-harvest days) and ambient temperature (°C) were the independent (or explanatory) variables of the study. The fruit freshness, color, shape, and weight (g) were the dependent (or response) variables of the study. The relationship between the fruit’s longitudinal and transverse diameter (i.e., L/T ratio) indicated the fruit shape [15].

Initially, a descriptive statistical analysis was performed to understand the overall behavior of the studied variables (see next section). The Pearson correlation (r) test was performed between variables (p < 0.01). This test served to verify the relationship (or not) between each pair of variables in a positive or negative way.

A multivariate analysis was also performed (in such method, not only temperature, but also fruit freshness, color, shape, weight, and L/T ratio, behave as independent variables). Then, a principal component analysis (PCA) was performed as a multivariate tool that can be used to visually identify cases suitable for further analysis with multivariate analysis algorithms [16]. Subsequently, a cluster analysis was also performed as an exploratory method of multivariate analysis [17]. The MINITAB® statistical software (version 14; PA, USA) was used to perform all the statistical analyses in the present study.

3. Results

It is instructive to highlight some descriptive statistical aspects of the ambient temperature behavior in this study (Table-1).

| Variable | Characteristics in the Study Period (17 Post-Harvest Days) |
|----------|----------------------------------------------------------|
| Temperature (°C) | Mean | Median | Mode | Variance | SD | Kurtosis | Min | Max |
| Temperature (°C) | 29.235 | 29.000 | 29.000 | 0.691 | 0.831 | -0.146 | 28.000 | 31.000 |
| Historical Mean Temperatures | 1901-1930 | 1931-1960 | 1961-1990 | 1991-2002 | Ref |
| 31.5 | 31.2 | 31.5 | 31.7 | [18] |

Note: SD, standard deviation; Min, minimum; Max, maximum; Ref, reference
It is important, on one hand, to point out that the mean temperature of the studied period was 29.24°C; thus, not exceeding the mean annual maximum temperatures of Manaus (AM, Brazil), recorded for more than a century (Table-1). On the other hand, the related kurtosis was less than 0.263 (-0.146), thus, a leptokurtic distribution (i.e., a more peaked curve, having fatter tails than a normal distribution of the same variance) [19].

The weight loss of green cubiu fruits did not constitute a continuous and drastic process in the post-harvest period at ambient atmosphere, but rather a gradual and paused process, as clearly detailed in Table-2 below.

Additionally, fruit weight versus post-harvest time has an inverse relationship, i.e., a negative correlation ($r = 0.96$). This can be clearly observed in the linear adjustment of the fruit weights in the post-harvest period (Figure-3).

### Table-2. Specifics of the Weight Loss of 20 Green Cubiu Fruits in the Post-Harvest Period at Ambient Atmosphere

| Post-Harvest Days | Fruit Mean Weight (FMW) | SD     | 95% CIs for FMW |
|-------------------|-------------------------|--------|-----------------|
| 1                 | 148.75                  | 31.91  | (133.82; 163.68) |
| 2                 | 148.75                  | 31.91  | (133.82; 163.68) |
| 3                 | 148.75                  | 31.91  | (133.82; 163.68) |
| 4                 | 146.25                  | 29.55  | (132.42; 160.08) |
| 5                 | 143.75                  | 30.21  | (129.61; 157.89) |
| 6                 | 141.25                  | 26.00  | (129.08; 153.42) |
| 7                 | 141.25                  | 26.00  | (129.08; 153.42) |
| 8                 | 141.25                  | 26.00  | (129.08; 153.42) |
| 9                 | 136.25                  | 23.61  | (125.20; 147.30) |
| 10                | 133.75                  | 26.00  | (121.58; 145.92) |
| 11                | 131.25                  | 24.16  | (119.94; 142.56) |
| 12                | 130.00                  | 23.79  | (118.87; 141.13) |
| 13                | 130.00                  | 23.79  | (118.87; 141.13) |
| 14                | 130.00                  | 23.79  | (118.87; 141.13) |
| 15                | 130.00                  | 23.79  | (118.87; 141.13) |
| 16                | 130.00                  | 23.79  | (118.87; 141.13) |
| 17                | 130.00                  | 23.79  | (118.87; 141.13) |

**Note:** SD, standard deviation; CIs, confidence intervals.

Nonetheless, according to the Pearson test (Table-3), a significant positive correlation ($p < 0.01$) was observed between the variables weight and longitudinal diameter, and weight and transverse diameter (implying that when the weight decreased, the longitudinal diameter and the transverse diameter also decreased). In addition, a positive relationship was noted between the variables longitudinal diameter and transverse diameter (i.e., when the longitudinal diameter decreased, the transverse diameter also decreased). The relationship between the variables L/T ratio and loss of freshness was also positive and significant ($p < 0.050$).

There was a significant negative correlation ($p < 0.01$) between the following variables, loss of freshness and weight, loss of freshness and longitudinal diameter, and loss of freshness and transverse diameter. In this case, there was an inversion between the variables, that is, when one variable decreased, the other increased. As a result, when the variables weight, longitudinal diameter, and transverse diameter decreased, there was an increase in the loss of freshness of the fruit and between the variables L/T ratio and transverse diameter. When the transverse diameter decreased, the L/T ratio increased. On the other hand, there was a negative correlation between the variables temperature and L/T ratio, and L/T ratio and weight (i.e., there was an inversion between the variables; when one increased the other one decreased).

### Table-3. Pearson Correlation Between Sensory and Physical Changes of Green Cubiu Fruits During the Post-Harvest Period at Ambient Atmosphere

|         | Temperature | Weight | L Φ | T Φ | L/T ratio |
|---------|-------------|--------|-----|-----|-----------|
| Weight  | 0.139(ns)   | 0.075(ns) | 0.329(ns) | -0.573(*) | -0.960(**) |
|         | (0.594)     | (0.775) | (0.197) | (0.016) | (0.994) |
| L Φ     | 0.942(**)   | 0.903(**) | 0.903(**) | -0.527(*) | -0.960(**) |
|         | (0.01)      | (0.01) | (0.01) | (0.030) | (0.001) |
| T Φ     | 0.898(**)   | 0.898(**) | 0.898(**) | -0.434(ns) | -0.911(**) |
|         | (0.001)     | (0.001) | (0.001) | (0.082) | (0.001) |
| L/T ratio | -0.786(**) | -0.786(**) | -0.786(**) | -0.879(**) | -0.879(**) |
|         | (0.001)     | (0.001) | (0.001) | (0.033) | (0.033) |

**Bold values are statistically significant**

**Note:** L Φ, longitudinal diameter; T Φ, transverse diameter; L/T ratio, longitudinal Φ/transverse Φ ratio; (ns), not significant at 5% level of probability; (*), significant at 5% level of probability; (**), significant at 1% level of probability; the values between parentheses correspond to $p$ values.
As briefly argued above, principal component analysis (PCA) and cluster analysis are both multivariate tools suitable for regrouping data and better visualizing their interrelationship, among other methodological advantages (e.g., correlative explanations, hypotheses).

The adequacy of the PCA was verified by the amount of information of the original variables, retained by two principal components (percentage of the total variation of the six variables accumulated by the first two components (Table-4).

Moreover, it was possible to explain all six variables of the present study with the two first principal components because from the fourth principal component on, there was a clear stabilization of the eigenvalue (Figure 4).

Table-4 also shows the two coordinates (axis 1 and axis 2) with the linear combination of six data matrix variables. The first axis contribution (4.2196) explained 70.3% of the question under study about four variables (weight, longitudinal diameter, transverse diameter, and loss of freshness) whereas the second axis contribution (1.3303) explained 22.2% of the question under study about the two other variables (temperature and L/T ratio), with a total inertia of 92.5%.

| Variables       | PC1        | PC2        |
|-----------------|------------|------------|
| Temperature     | 0.142      | 0.775      |
| Weight          | 0.465      | -0.180     |
| L Ф             | 0.449      | -0.245     |
| T Ф             | 0.480      | 0.067      |
| L/T ratio       | 0.353      | -0.483     |
| Freshness loss  | -0.454     | 0.263      |
| Eigenvalue      | 4.2196     | 1.3303     |
| Proportion (%)  | 70.3       | 22.2       |
| Cumulative (%)  | 70.3       | 92.5       |

Bold values are statistically significant
Note: L Ф, longitudinal diameter; T Ф, transverse diameter; L/T ratio, longitudinal Ф/transverse Ф ratio

Also in the analysis of the main components (Table-4), the elements that contributed the most to the present results were the weight (0.465), the longitudinal diameter (0.449), and the transversal diameter (0.480).

As briefly explained above, PCA aims at visualizing the proximity between individuals and the relations between the variables to achieve a graphical summary of the data set (Figure 5).

The aim of Figure-6 was to find the plane that best represents the cloud of points, so that it was possible to see the proximity between the points. Additionally, in this figure, the post-harvest days were arranged from right to left.

Figure-7 shows the results of PCA and cluster analysis. The 17 post-harvest days were gathered in four clusters (or groups) as follows:

- Group 1, from the first to the sixth day;
- Group 2, the seventh day;
- Group 3, from the eighth to the ninth day;
- Group 4, from the tenth to the seventeenth day;

This clustering was based on the correlations of the six variables thoroughly investigated in this study (i.e., temperature, fruit freshness, color, shape, weight, and L/T ratio).

Figure-8 shows (from left to right), (i) vigorous green cubiu fruits on the first experiment day, (ii) a column chart with absolute and percentage values of the gradual loss of freshness of the fruits over the 17 days of the experiment, and (iii) the total loss of freshness of all fruits as well as their imperfect color changes on the last experiment day.

This figure also shows that cubiu fruits harvested at the green stage do not present the characteristic progression of the peel colors towards changes compatible with other ripening stages (in fact, in this study, no fruit reached the fully ripe stage, distinctive by its beautiful dark red or wine color, as mentioned before).

At the end of the experiment, all fruits were cut (see 2. Materials and Methods) to evaluate the internal integrity of the tissues, and the most prominent finding was the placental (endocarp) abruption (Figure-9).

4. Discussion

Post-harvest fruit freshness is an important research topic for the fruit industry in general, including that of cubiu fruits (in particular) [20]. The loss of cubiu fruit freshness started on the sixth post-harvest day (one fruit out of 20), being apparent in 100% of the fruits 17 days later (end of the experiment). Nonetheless, this experiment also demonstrated that cubiu fruits harvested at the green stage are more resistant to the ambient atmosphere than other members of the Solanaceae family actually are. For instance, the smaller fruits of hot pepper (Capsicum frutescens L.) need immediate conservation methods (e.g., commercialization in brine) whereas tomato fruits (Solanum lycopersicum L.) start losing freshness at the second post-harvest day at high temperatures [21].

Cubiu moisture content is a major factor in determining the appearance of freshness of the fruit, and it has been extensively studied in previous works in whole cubiu fruits as well as in all the tissue portions of the fruit (i.e., peel, pulp, and placenta) at four ripening stages (i.e., green, turning, ripe, and fully ripe stages) [4] [5] [10]. Additionally,
water is the most prominent macronutrient of cubiu fruits with a positive Pearson correlation \((r = 0.872, p < 0.05)\) with the mean weight of the fruits during ripening [4]. Cubiu peel exhibits an overall moisture lower than that of the pulp (the highest moisture) and the placenta (an intermediary moisture) during ripening [4]. However, the loss of moisture during the post-harvest period at ambient atmosphere cannot be attributed as the sole cause of placental abruption in the fruits herein studied; cubiu placenta itself being, overall, poorly studied and known.

Temperature is the environmental (abiotic) factor that most clearly influences the deterioration rate of harvested commodities such as fresh fruits (e.g., for each increase of 10°C above optimum, the rate of deterioration increases by two-to threefold) [22]. In this study, there was a statistically significant negative correlation between the temperature and the L/T ratio. Additionally, the predominant shape of cubiu fruits was cordiform (1.17) throughout the experiment, i.e., a fruit form yet suitable to easily peel.

In general, fruit weight loss is associated with respiration and water evaporation through the peel, and this alteration is inevitable with prolonged storage, even in the cold [23] [24]. Similarly, in general terms, fruits are classified according to the respiratory pattern in climacteric fruits (rapid increase of oxygen consumption and ethylene production, with biochemical transformations responsible for plant ripening or other post-harvest changes) and non-climacteric fruits (without respiratory variation, nor metabolic, with maturation in the mother plant) [7] [25]. Fruits of the Solanaceae family such as tomatoes are classified as climacteric, others, such as eggplant \((Solanum melongena L.)\) and pepper, as non-climacteric [7] [25]. However, there are fruits such as guava \((Psidium guajava L.)\) from the Myrtaceae family, or the abovementioned pepper, which present both patterns (climacteric and non-climacteric) of respiration, depending on the cultivar or genotype [25]. There are works demonstrating that cubiu fruits may also exhibit both respiratory patterns [26] [27]. Thus, a possible non-climacteric pattern of the 20 fruits herein studied would explain their absence of ripening.

The ideal way to measure the maturity of a fruit should be simple, fast, feasible in the planting area itself and, if possible, without rendering the fruit unattractive to potential consumers [28]. In practical terms, mature and ripe fruit can be distinguished by the following description: mature fruit is ready for consumption whereas ripe fruit is ready for picking [6]. As stated before, colors are indicators of the ripening stages of fruits [7] [10]. However, in this experiment, the color changes started on the seventh post-harvest day at ambient atmosphere, but they were characteristically irregular, imperfect, and incompatible with three standard ripening stages of the fruit at the experimental end (i.e., eight fruits (40%) remained green, seven fruits (35%) reached the turning stage, and five fruits (25%) reached the ripe stage). Furthermore, at the end of this experiment, no fruit reached the fully ripe stage.

Thus, freshness enhances the colors of cubiu fruits, and these two sensory indices are taken into account in the pertinent market transactions.

5. Conclusion
This observational study sought to understand the sensory and physical changes that occur in perfect cubiu fruits harvested at the green stage and stored at ambient atmosphere. Despite the daily careful measurements of the investigated variables, the analysis of the data was complex (especially regarding the fruit weight), although it was possible to demonstrate some significant correlations already discussed. Future investigations in similar experimental conditions, but focusing on macronutrient and micronutrient variations (as well as on other cubiu metabolites), will further clarify these multifarious changes.

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Figures

Figure 1. Centered view of a cubiu shrub with fruits at different ripening stages.

Figure 2. Factors affecting fruit weight:
- Fruit size, seed weight
- Chemical treatment (e.g., borax)
- Fruit thinning, increased irrigation
- Fruit water accumulation during ripening
- Phytohormones (e.g., GA3)
- Macronutrients (e.g., K, P, Ca, Mg)
- Nitrogen (key factor)
**Figure-3.** Formation of plateaus in the linear adjustment of cubiu fruit weights (g) as a function of the 17 post-harvest days.

**Figure-4.** Composition of the number of principal components by means of the maximum variances.
Figure 5. Correlation between the variables represented in the factorial plane of the axes 1 and 2

Figure 6. Representation of the 17 post-harvest days in the factorial plan of the axes 1 and 2
Figure 7. Clustering of 17 post-harvest days based on the dendrogram analysis of the six investigated variables.

Figure 8. Absolute and percentage values of the gradual loss of freshness of the fruits over the 17 days of the experiment.
Figure 9. Axial abruption of cubiu placenta observed at the 17th post-harvest day.

Note: Arrow (A) shows the empty space created by the placental abruption. Arrow (B) shows the remaining tissue of the pulp, highlighting the retraction of the multilocular space.