

Nutrients of Cubiu Fruits (*Solanum sessiliflorum* Dunal, Solanaceae) as a Function of Tissues and Ripening Stages

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Abstract Cubiu fruits (*Solanum sessiliflorum* Dunal, Solanaceae) are nutritionally dense. However, most studies have investigated cubiu nutrients in whole fruits. This study evaluated cubiu fruit nutrients as a function of different tissues (peel, pulp, and placenta) and ripening stages (green, turning, ripe, and fully ripe). Fruits ($n = 118$) were harvested to investigate weight, macronutrients (moisture, ash, proteins, lipids, carbohydrates, and energy) and selected macrominerals-calcium (Ca), magnesium (Mg), phosphorus (P), and potassium (K). Analyses were performed at the *Instituto Nacional de Pesquisas da Amazônia (INPA)*, Brazil. Macronutrient results were expressed in g/100 g fresh weight, macrominerals as g/kg fresh weight. Fruit weight increased throughout ripening. Moisture reached a maximum value (91.77) in the fully ripe pulp. Ash displayed a maximum value (0.86) in the fully ripe placenta. Energy reached a maximum value (356.66 kJ, 86.57 kcal) in the fully ripe placenta. Carbohydrates decreased from the peel to the placenta (lowest values). K exhibited a maximum value (42.257) in the green pulp. The P peaked in ripe pulp (24.450) and placenta (21.240). In the ripe placenta, Ca had a maximum value (7.860) followed by Mg (7.573). This detailed knowledge of the edible parts of ripening cubiu fruits will broaden their use in health and disease.

Keywords: chronic kidney disease (CKD), diabetes mellitus, lipids, obesity, phosphorus, potassium, proteins

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1. Introduction

Cubiu (*Solanum sessiliflorum* Dunal), also known as cocona, is a fruit shrub native to the western Amazonia. It belongs to the Solanaceae family and has been domesticated by the pre-Columbian Amerindians for centuries. Cubiu fruits are highly appreciated in Brazil and other countries (e.g., Colombia, Peru, Bolivia, and Venezuela) especially for their organoleptic characteristics (vivid colors, juiciness, acidity, gelling properties, typical aroma, and unique flavor). It is used for many delicious recipes. Nevertheless, cubiu fruits are also appreciated for their complex biology, nutritional richness, and phytochemical potential, and it is important to review these aspects in more detail.

Cubiu shrubs have drawn the attention of researchers for their biological versatility (preferential heliophilous or facultative ombrophilous plants), their capacity to grow in upland or lowland areas, their climacteric (ethylene-dependent) as well as non-climacteric (ethylene-independent) behavior (depending upon the cultivar or the genotype), and the good technological quality of their fruits for the food industry [1].

During ripening, cubiu fruits acquire vivid and distinctive colors due to the tissue predominance of

different pigments. Briefly, chlorophylls *a* and *b* decline during cubiu fruit ripening whereas total flavonoids and total carotenoids increase at the final stages [1]. In ripe cubiu fruits, the major carotenoids are β -carotene and lutein [2]. Chlorophylls are phytochemicals associated with various potential benefits to human health [1]. Phytonutrients are plant nutrients with specific biological activities that support human health, e.g., carotenoids, such as β -carotene, have provitamin A activity, and flavonoids may present numerous potential benefits to human health [1,3].

Cubiu fruits are consumed at all ripening stages and once manually harvested, carefully washed, and dried, they may be stored at the ambient temperature (e.g., $\pm 29^{\circ}\text{C}$) and remain fit for consumption for five days. Many methods may be applied to extend cubiu's shelf life, but this discussion is beyond the scope of this study.

Cubiu fruits are generally classified into large and small fruits, but their phenotypic variation is more complex and involves more parameters as discussed below. Cubiu fruits have a histology similar to tomato (*Solanum lycopersicum* L., Solanaceae), i.e., thin peel (exocarp), fleshy pulp (mesocarp), and axial placenta (endocarp); they are often described as the Indian tomato [1,2].

Cubiu fleshy berries are characteristically juicy, and the pulp moisture usually exceeds 90% [1,4]. In cubiu fruits,

there is a considerable increase in the organic acid content (e.g., citric and malic acids) throughout ripening—especially in the placenta [1,5]. The gelling properties of cubiu fruits are associated with their rich content in pectin, particularly in the fully ripe pulp [1,6,7]. Importantly, this soluble dietary fiber is a functional food ingredient that may reduce glucose absorption, exert hypocholesterolemic effect, delay gastric emptying (also enhancing satiety), and act as a prebiotic [8].

Fresh cubiu fruits have a typical and pleasant aroma. The flavor of cubiu fruits is very acidic with a small degree of sweetness [6]. Interestingly, both cubiu peel and pulp undergo dehydration for the manufacture of dry cubiu peel and flour. These products have a pleasant bittersweet taste similar to tamarind fruit (*Tamarindus indica* L., Fabaceae).

Except for the moisture mentioned above, fresh whole cubiu fruits are relatively poor in other macronutrients including at the ripe stage (g/100 g): ash (0.4), proteins (0.7), lipids (0.3), dietary fibers (1.6), carbohydrates (5.2), and energy (124.20 kJ, 29.50 kcal) [9]. On the other hand, fresh cubiu fruits are rich in vitamin C (or L-ascorbic acid) in the peel, pulp, and placenta throughout ripening. They reach a maximum value in the fully ripe peel (32.45 mg/100 g fresh weight) [1]. Different parts of the cubiu have different amounts of macrominerals and microminerals [10,11].

The concept of energy-dense fruits is self-limited to calories, whereas that of nutrient-dense fruits is broader and comprises other functional food constituents including essential (or indispensable) amino acids [9]. This allows cubiu fruits to be characterized as nutritionally dense and low in calories [9-11]. Plant proteins are usually less valuable than animal proteins due to the lack of indispensable amino acids [12,13]. However, this is commonplace and is not applicable to cubiu fruits whose indispensable amino acid composition is complete [5].

The most abundant nonessential (or dispensable) amino acids in cubiu fruits (in descending order) are asparagine, serine, and glutamine [5]. Asparagine is necessary for healthy brain development in children [14]. Serine is also necessary for the biosynthesis of ceramides—the precursors of sphingolipids and phosphatidylserine [15]. Glutamine is the most abundant amino acid in human plasma and participates in many metabolic pathways required for normal cell function [16].

Along with secondary metabolites such as flavonoids and carotenoids, phenolic compounds (e.g., 5-caffeoylquinic acid, eugenol, and methyl salicylate) are among the cubiu fruit functional constituents [1,2,5]. The 5-caffeoylquinic acid is a major phenolic compound that may exert antioxidative, antitumor, and anti-inflammatory activities [2,17]. Eugenol occurs in many other plants (e.g., clove, *Dianthus caryophyllus* L., Caryophyllaceae) and may exert a wide range of biological activities (e.g., analgesic, anti-inflammatory, antibacterial, antifungal, antiviral, and antihypertensive) [18]. Methyl salicylate is a phenolic compound that serves in plant defenses against pathogens and herbivores [19]. It has anti-inflammatory effects [20].

The Solanaceae family and its genus *Solanum* L., in particular, are diversified in a class of secondary metabolites called glycoalkaloids. In addition to their relevance

as naturally occurring anti-nutrients (e.g., α -solanine in potatoes (*Solanum tuberosum* L.) and α -tomatine in tomatoes), these compounds are not only involved in plants' defense responses, but they have also considerable medicinal potential and promising applications in human health [21]. For instance, green fruits of *Solanum* species have high amounts of solasodine (an anticancer glycoalkaloid): *S. paludosum* Moric. (0.75%), *S. asperum* Rich. (0.67%), and *S. sessiliflorum* Dunal (0.30%) [22,23].

With such an arsenal of phytonutrients (e.g., flavonoids, carotenoids, and polyphenols) and other beneficial molecules (e.g., solasodine), cubiu is a promising solanaceous fruit with great potential health benefits. However, there has been no previous study on cubiu macronutrients that included the fruit placenta nor has the impact of ripening been studied. Thus, the aim of this study was to evaluate the weight and the macronutrients of cubiu fruits (i.e., moisture, ash, proteins, lipids, carbohydrates, and energy) as well as selected macrominerals (i.e., calcium (Ca), magnesium (Mg), phosphorus (P), and potassium (K)), according to different tissue portions (i.e., peel, pulp, and placenta) and ripening stages (i.e., green, turning, ripe, and fully ripe).

2. Materials and Methods

2.1. Sample Preparation

Similar to previous work [1], cubiu fruits were randomly and manually harvested from plants grown in low humic gley soil at the upland experimental farm of Universidade Federal do Amazonas (UFAM) in the municipality of Manaus, Brazil (2°39'1.98" south and 60°3'18.41" west, 67 m altitude). The determination of the ripening stages of the fruits (i.e., green, turning, ripe, and fully ripe stages) was based on the four-color pattern described by the Amazonian population of Brazil (Figure 1). In addition to the standard colors, a supplementary criterion for the inclusion of the fruits in this study was their structural integrity (i.e., absence of injuries). After harvest, the fruits were transported in plastic containers to the laboratory of the Department of Food Technology of *Coordenação de Tecnologia e Inovação (COTI) of Instituto Nacional de Pesquisas da Amazônia (INPA)*, Manaus, Amazonas, Brazil, where the fruits were washed and dried with a paper towel at ambient temperature (22°C).

The sample size ($n = 118$) of whole cubiu fruits at four ripening stages (the independent variables of this study) included 41 green fruits, 26 turning fruits, 29 ripe fruits, and 22 fully ripe fruits. The fruits were then weighted, and the peel, pulp, and placenta from the four ripening stages were carefully separated and homogenized in the blender (BLSTVB-RVO-000, Oster, USA). Some of the homogenized material was immediately used to determine moisture (and dry matter). These were the dependent variables. The remaining part of the homogenized material was stored in polyethylene bags and kept in a freezer (−20°C) until use in the analyses of other dependent variables of this study (i.e., lipids, proteins, ash, and selected macrominerals—Ca, Mg, P, and K). Three replicates were prepared from each sample.

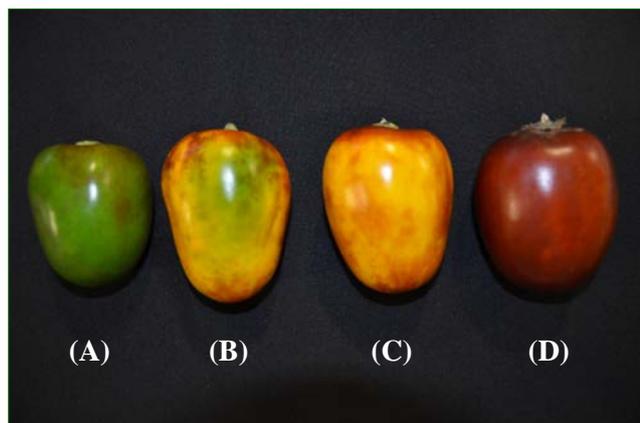


Figure 1. Cubiu fruits at four standard ripening stages. Green stage (A), turning stage (B), ripe stage (C), and fully ripe stage (D). Cubiu peel color is more homogeneous at the green and fully ripe stage whereas it is prone to more variations at the turning and ripe stage.

2.2. Fruit Weight

Cubiu fruits were separated from the total sample above described and regrouped according to the color patterns of their respective ripening stages, being subsequently counted and weighed [24].

2.3. Pulping of Cubiu Fruits

According to the previous description [1], each cubiu fruit was cut transversely with a stainless steel knife so that the seeds were preserved. Fruit tissue portions (*i.e.*, peel, pulp, and placenta) were manually separated. The pulp that adhered to the peel was carefully removed with a spatula. As with other Solanaceae species, cubiu fruits present an axial placentation that facilitates the removal of this tissue portion.

2.4. Moisture and Dry Matter

Similar to the methodology previously described [24], the tissue portions of freshly harvested cubiu fruits were homogenized in the blender and immediately kept in a furnace (with forced air circulation) at 65°C until a constant weight was reached within 12 hours. The dry matter (DM), also referred to as total solids, was estimated by difference.

2.5. Lipids

Lipids of dry homogenized portions of cubiu fruit tissues were extracted with hexane (Biotec, Pinhais, Brazil) by the traditional Soxhlet method [24,25]. Afterwards, lipids were expressed in g/100 g fresh weight.

2.6. Proteins

Proteins were determined by the micro-Kjeldahl digestion process to quantify nitrogen content [24]. The nitrogen released by the decomposition of dry organic matter was transformed into ammonia (NH₄OH). This compound, in the presence of boric acid (H₃BO₃), could be titrated with a solution of hydrochloric acid (HCl) of known normality. In the final calculation, the empirical factor 6.25 was

introduced to convert the number of grams of nitrogen found in the samples into the number of grams of proteins. Proteins were expressed in g/100 g fresh weight.

2.7. Ash

This analytical procedure consisted of the carbonization of dried portions of cubiu tissues [24]. The dried portions were then burned at 550°C in a muffle furnace (EDGCON 1P, São Paulo, Brazil) for 4 hours, and the total residual ash was measured [24]. Ash was expressed in g/100 g fresh weight.

2.8. Carbohydrates

The carbohydrate content was estimated by the difference of the average values of the other measured macronutrients [24]. Carbohydrates were expressed in g/100 g fresh weight.

2.9. Energy Content of Fruit Tissue Portions

The energy content was expressed both in kilojoules (kJ) and the corresponding kilocalories (kcal) per 100 grams of fresh fruit tissue (*i.e.*, peel, pulp, or placenta) using the respective conversion factors 37 kJ (9 kcal) for lipids and 17 kJ (4 kcal) for proteins and carbohydrates [9,24].

2.10. Selected Macrominerals (Ca, Mg, P, and K) of Cubiu Fruit Tissues

The method used to extract minerals from plant tissues is known as nitroperchloric digestion. Sample preparation uses concentrated nitric acid (HNO₃) and concentrated perchloric acid (HClO₄) at a 3:1 ratio. This is highly oxidizing and dissolves all plant tissues and releases all nutrients in the sample into solution. This method effectively prepares plant tissues for quantitative determination of the concentration of macrominerals such as Ca, Mg, P, and K and uses acid digestion in conjunction with external heating [26].

For cubiu fruit digestion, 0.5 g of ground tissue portions was weighed using butter paper. Subsequently, 8 mL of the nitroperchloric solution was added to the tubes with the plant materials. This pre-digestion process was performed on the day before the actual digestion at the end of the day (\pm 5 p.m.) and left overnight. During the actual digestion process, the samples were placed in the digestion blocks (in a chemical hood) with a starting temperature of 50°C. This temperature was maintained for 30 minutes after which it was gradually raised to 100°C and held for a further 30-minute period. Next, the temperature was raised to 150°C until the total organic matter vanished (appearance of reddish fumes) within 30 minutes. After raising the temperature to 210°C (final temperature) until the appearance of white fumes, the extract became colorless and the final volume reached \pm 1 mL. The samples were removed from the digestion blocks and appropriately cooled in a chemical hood. After complete cooling, the extract was transferred to the 50 mL volumetric flask via a funnel, and the volume was completed with distilled water. The extract was then stored in a dark flask.

The K of the aqueous solution could be aspirated and quantitated using a flame photometer [27,28]. To measure P, the diacid ion H_2PO_4 in a strongly acidic medium reacted with molybdate (MoO_4^{2-}) to form a blue staining complex; the intensity of this coloration was proportional to the concentration of P in the UV-VS spectrophotometer. The Ca and Mg were read at 422.7 nm and 285.2 nm, respectively, in an atomic absorption spectrophotometer with an air-acetylene flame. Ca, Mg, P, and K were then expressed in g/kg fresh weight.

2.11. Statistical Analyses

The experiments were conducted in a completely randomized design comprising three tissue portions of cubiu fruits (peel, pulp, and placenta) at four ripening stages (green, turning, ripe, and fully ripe) [1]. There were three replicates from each investigated sample. Initially, the analysis of variance (ANOVA) was performed for each of the variables [1]. These were verified by the *F* test and in case of differences, by the Tukey test (both tests with 5% probability) [1]. Subsequently, the Pearson correlation (*r*) was performed between variables ($p < 0.05$) using MINITAB statistical software (version 14; PA, USA). The results were expressed as mean values followed by standard deviations.

3. Results

3.1. Cubiu Fruit Weight during Ripening

The results for the descriptive statistics of cubiu fruit weight during ripening are summarized in Table 1. Cubiu fruits had a variable weight. The amplitude of weight variability was important for green (103.98), turning (122.58), ripe (120.78), and fully ripe (133.95) cubiu fruits. The mean weight (g) increased over the course of ripening, *i.e.*, 119.39, 144.50, 184.62, and 196.80 at the green, turning, ripe, and fully ripe stage, respectively. Apart from the absence of modal values, all characteristics of the fruit weight were concordant in this study and described increments as ripening progressed.

Table 1. Descriptive Statistics of the Weight (g) of 118 Cubiu Fruits at Four Ripening Stages

Characteristics	Ripening Stages			
	Green	Turning	Ripe	Fully Ripe
Total	41	26	29	22
Minimum	71.24	77.00	107.55	139.38
Maximum	175.22	199.58	228.33	273.33
Mean	119.39	144.50	184.62	196.80
First quartile	102.09	125.91	160.72	165.15
Median	117.52	143.99	188.41	188.20
Third quartile	135.58	160.51	210.28	238.74
Mode	Amodal	Amodal	Amodal	Amodal
Variance	565.18	719.73	982.63	1675.83
SD ^a	23.77	26.83	31.35	40.94
LL CI ^b (95%)	111.88	133.67	172.70	178.65
UL CI ^c (95%)	126.89	155.34	196.54	214.95

^aSD: Standard deviation. ^bLL CI: Lower limit of confidence interval. ^cUL CI: Upper limit of confidence interval.

3.2. Selected Macrominerals (Ca, Mg, P, and K) of Cubiu Fruit Tissues during Ripening

Table 2, Table 3 and Table 4 show the results of selected macrominerals of cubiu fruit tissues during ripening. The K and P were the most abundant macrominerals (followed by Ca) in cubiu fruits. K reached a maximum value (42.257 g/kg fresh weight) in cubiu pulp at the green stage. P was highest in the pulp (24.450 g/kg fresh weight-*p* value < 0.05) and in the placenta (21.240 g/kg fresh weight-*p* value > 0.05) of ripe cubiu fruits. Apart from these two peaks, P was lower than the other macrominerals in all tissues throughout ripening. The Ca showed a maximum value (7.860 g/kg fresh weight) in the placenta at the ripe stage followed by Mg (7.573 g/kg fresh weight) in the same tissue portion and ripening stage. The cubiu pulp and placenta had the most macrominerals.

Table 2. Selected Macrominerals of Cubiu Peel during Ripening.

Stages ^a	Peel macrominerals ^{b,c} (g/kg fresh weight)			
	(K)	(P)	(Ca)	(Mg)
(G)	24.787	1.583	5.013	4.983
	0.543 a	0.119 a	0.095 a	0.258 a
(T)	15.197	1.083	2.870	2.963
	2.921 a	0.050 c	0.248 b	0.571 b
(R)	22.923	1.367	3.447	3.250
	11.454 a	0.038 b	0.490 b	0.350 b
(FR)	16.793	1.383	3.537	3.127
	1.372 a	0.050 b	0.325 b	0.207 b

^a(G): Green; (T): Turning; (R): Ripe; (FR): Fully ripe. ^b(K): Potassium; (P): Phosphorus; (Ca): Calcium; (Mg): Magnesium. ^cMeans and standard deviation followed by the same letter did not significantly differ between variables at the level of 5% probability by the Tukey test.

Table 3. Selected Macrominerals of Cubiu Pulp during Ripening

Stages ^a	Pulp macrominerals ^{b,c} (g/kg fresh weight)			
	(K)	(P)	(Ca)	(Mg)
(G)	42.257	3.187	4.373	4.137
	1.455 a	0.101 b	0.081 b	0.248 b
(T)	26.007	2.500	3.417	3.353
	1.385 c	0.225 b	0.595 c	0.696 b
(R)	31.803	24.450	5.503	5.430
	1.521 b	18.139 a	0.367 a	0.241 a
(FR)	34.233	3.603	5.790	5.557
	0.801 b	0.200 b	0.190 a	0.168 a

^a(G): Green; (T): Turning; (R): Ripe; (FR): Fully ripe. ^b(K): Potassium; (P): Phosphorus; (Ca): Calcium; (Mg): Magnesium. ^cMeans and standard deviation followed by the same letter did not significantly differ between variables at the level of 5% probability by the Tukey test.

Table 4. Selected Macrominerals of Cubiu Placenta during Ripening

Stages ^a	Placenta macrominerals ^{b,c} (g/kg fresh weight)			
	(K)	(P)	(Ca)	(Mg)
(G)	29.767	4.210	6.817	6.093
	7.837 a	0.400 a	0.845 b	0.495 c
(T)	31.990	4.110	6.517	6.503
	3.496 a	0.080 a	0.160 b	0.160 bc
(R)	27.247	21.240	7.860	7.573
	9.442 a	27.900 a	0.305 a	0.217 a
(FR)	35.697	5.070	7.167	6.860
	3.916 a	0.570 a	0.081 ab	0.110 b

^a(G): Green; (T): Turning; (R): Ripe; (FR): Fully ripe. ^b(K): Potassium; (P): Phosphorus; (Ca): Calcium; (Mg): Magnesium. ^cMeans and standard deviation followed by the same letter did not significantly differ between variables at the level of 5% probability by the Tukey test.

3.3. Macronutrient and Caloric Content of Cubiu Fruit Tissues during Ripening

Table 5, Table 6 and Table 7 display the macronutrient content and Table 8 the caloric content of cubiu fruit tissues according to different tissue portions (*i.e.*, peel, pulp, and placenta) and four ripening stages (*i.e.*, green, turning, ripe, and fully ripe).

Table 5. Macronutrient Content of Cubiu Peel during Ripening

Stages ^a	Macronutrients ^{b,c} (g/100 g fresh weight)					
	(M)	(DM)	(A)	(P)	(L)	(C)
(G)	88.31 0.08 a	11.69	0.42 0.03 a	0.80 0.06 b	0.45 0.02 a	10.02
(T)	86.65 0.36 b	13.35	0.36 0.07 ab	0.72 0.02 b	0.10 0.00 b	12.17
(R)	85.27 0.35 c	14.73	0.32 0.01 b	0.91 0.05 bc	0.09 0.00 bc	13.41
(FR)	83.15 0.83 d	16.85	0.45 0.01 a	1.10 0.08 a	0.07 0.00 c	15.23

^a(G): Green; (T): Turning; (R): Ripe; (FR): Fully ripe. ^b(M): Moisture; (DM): Dry matter; (A): Ash; (P): Proteins; (L): Lipids; (C): Carbohydrates. ^cMeans and standard deviation followed by the same letter did not significantly differ between variables at the level of 5% probability by the Tukey test.

Table 6. Macronutrient Content of Cubiu Pulp during Ripening.

Stages ^a	Macronutrients ^{b,c} (g/100 g fresh weight)					
	(M)	(DM)	(A)	(P)	(L)	(C)
(G)	91.75 0.26 a	8.25	0.51 0.03 a	0.52 0.00 a	1.01 0.00 b	6.21
(T)	91.52 0.23 a	8.48	0.57 0.05 a	0.47 0.01 b	0.70 0.26 b	6.74
(R)	91.39 0.32 a	8.61	0.57 0.02 a	0.49 0.01 c	1.23 0.04 ab	6.32
(FR)	91.77 0.01 a	8.23	0.61 0.09 a	0.43 0.01 d	1.56 0.03 a	5.63

^a(G): Green; (T): Turning; (R): Ripe; (FR): Fully ripe. ^b(M): Moisture; (DM): Dry matter; (A): Ash; (P): Proteins; (L): Lipids; (C): Carbohydrates. ^cMeans and standard deviation followed by the same letter did not significantly differ between variables at the level of 5% probability by the Tukey test.

Table 7. Macronutrient Content of Cubiu Placenta during Ripening

Stages ^a	Macronutrients ^{b,c} (g/100 g fresh weight)					
	(M)	(DM)	(A)	(P)	(L)	(C)
(G)	90.68 0.27 a	9.32	0.54 0.02 b	1.11 0.01 a	3.58 0.12 d	4.09
(T)	89.34 0.65 ab	10.66	0.77 0.03 a	1.10 0.02 a	4.55 0.15 c	4.24
(R)	89.15 0.42 bc	10.85	0.85 0.07 a	1.12 0.02 a	6.32 0.22 b	2.56
(FR)	88.76 0.73 c	11.25	0.86 0.05 a	1.11 0.02 a	9.01 0.15 a	0.26

^a(G): Green; (T): Turning; (R): Ripe; (FR): Fully ripe. ^b(M): Moisture; (DM): Dry matter; (A): Ash; (P): Proteins; (L): Lipids; (C): Carbohydrates. ^cMeans and standard deviation followed by the same letter did not significantly differ between variables at the level of 5% probability by the Tukey test.

Table 8. Caloric Content of Cubiu Fruit Tissues during Ripening

Stages ^a	Peel		Pulp		Placenta	
	(kJ) ^b	(kcal) ^c	(kJ) ^b	(kcal) ^c	(kJ) ^b	(kcal) ^c
(G)	200.59	47.33	151.78	36.01	220.86	53.02
(T)	222.83	52.46	148.47	35.14	259.13	62.31
(R)	246.77	58.09	161.28	38.31	296.40	71.60
(FR)	280.20	65.95	160.74	38.28	356.66	86.57

^a(G): Green; (T): Turning; (R): Ripe; (FR): Fully ripe. ^b(kJ): Kilojoules. ^c(kcal): Kilocalories.

Water was 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. Cubiu peel exhibited an overall moisture lower than that of the pulp (the highest moisture) and the placenta (an intermediary moisture) during ripening. The cubiu peel moisture decreased gradually from the green stage to the fully ripe stage. The carbohydrate content of cubiu peel was particularly high and had a consequent increase in the caloric content of this tissue. In addition, the dry matter of cubiu fruit peel increased more significantly from the green stage to the fully ripe stage due to the high carbohydrate content of this tissue. Peel proteins showed the maximum value (1.10 g/100 g fresh weight) at the fully ripe stage. This also corresponded to the maximum caloric value (280.20 kJ, 65.95 kcal) of this tissue.

Cubiu pulp dry matter was remarkably stable throughout ripening as was the ash content of the cubiu pulp. This formed a plateau with a modal value (0.57 g/100 g fresh weight) at the turning and ripe stage. This was also seen at the fully ripe stage. On the contrary, the pulp protein content of cubiu fruit decreased slightly during ripening. The pulp lipid content of cubiu fruit clearly increased and reached higher values at the ripe (1.23 g/100 g fresh weight) and the fully ripe stage (1.56 g/100 g fresh weight). The carbohydrate content of cubiu pulp remained stable from the green to the ripe stage. It decreased at the fully ripe stage.

The ash content of cubiu placenta increased throughout ripening and reached maximum values (0.86 g/100 g fresh weight) at the fully ripe stage. The dry matter of cubiu placenta increased during ripening due to the equally increasing contents of proteins and lipids. Cubiu placenta had a stable protein content with one modal value (1.11 g/100 g fresh weight) at the green and fully ripe stages. Furthermore, the tendency of high lipid content already noticed in cubiu pulp was confirmed in cubiu placenta with higher and increasing values throughout ripening. Notably, the lipid predominates over the other macronutrients in cubiu placenta. This became evident during ripening. Placenta was the most energy-dense and nutrient-dense tissue of cubiu fruits throughout ripening.

4. Discussion

4.1. Cubiu Fruit Weight during Ripening

The results regarding cubiu fruit weight during ripening agree well with a previous study in which the cubiu fruit has a variable weight partially due to genetic factors [24]. Nevertheless, other than the genetic factors, there are many other factors that might increase the fruit weight such as pruning, thinning, increased irrigation, fruit water accumulation during the enlargement of mesocarp cells, nitrogen, urea, macromineral availability (*e.g.*, especially of K and P, but also of Ca and Mg), and phytohormones (*e.g.*, gibberellic acid or gibberellin) [29-42]. Some of these factors are obviously interrelated, but many may also be confounding.

Multifactorial inheritance is defined as traits resulting from the interplay of multiple environmental factors (*e.g.*, pedoclimatic factors) with multiple genes (or polygenes)

[43,44,45,46]. Fruit weight is a quantitative trait influenced by the combined action of polygenes and environmental factors [44,46,47]. Large edible fruits such as cubiu result from selection, domestication, and long breeding practices for desirable traits (e.g., higher weight) [1]. As a result, the cubiu fruit mean weight may reflect phenotypic stability [11].

This study had a larger sample size than prior work and confirmed the previous finding of an increasing mean weight of cubiu fruits during ripening [24]. Importantly, this study also showed a positive Pearson correlation between the moisture content and the mean weight of cubiu fruits. Juicy fruits such as cubiu are important water sources. However, cubiu weight as well as cubiu width and length correlate with the moisture content of the fruit [48]. Large ripe cubiu fruits usually exceed 200 g [24]. In this study, the maximum fruit weight (273.33 g) was reached at the fully ripe stage. This high weight is one of the main characteristics attracting consumers to cubiu fruits.

Quantitative trait loci (QTL) are measurable regions of the genome affecting highly polygenic traits such as fruit weight, sugar content, and acidity [49,50]. QTL associated with fruit weight has aroused great interest in different breeding programs [44]. QTL may also advance understanding of phenotypic variations in cubiu fruits.

4.2. Selected Macrominerals (Ca, Mg, P, and K) of Cubiu Fruit Tissues during Ripening

Macrominerals are necessary in large amounts for both plants and human beings [12,51]. Minerals should be found in fresh fruits and vegetables [52]. The Ca, Mg, P, and K are essential for the entire cubiu plant including leaves, stems, and roots as demonstrated in a well-conducted study on the omission of these macrominerals (among others) in cubiu nutrition with the subsequent appearance of deficiency symptoms (e.g., chlorosis—a yellowing of normally green leaf tissue) [53,54].

Macrominerals in fruits may be base-forming elements (e.g., Ca, Mg, and K) and acid-forming elements (e.g., P) [55,56]. The literature shows high contents of P in the pulp of cubiu fruits [4,5,10,57]. However, P exhibited a significant peak in the pulp and in the placenta of ripe cubiu fruits. The presence of high levels of P is of interest not only because cubiu fruits are usually consumed at the ripe stage, but also because the pulp and the placenta are the most commonly consumed parts of cubiu. The high K content is often associated with increased acidity, and cubiu fruits are quite acidic [1,6,55]. High K content is also associated with improved fruit color (Figure 1), and a high Ca content reduces the incidence of physiological disorders and improves the quality of fruits [55]. Although cubiu fruit Ca and Mg values were not high in this study, they evolved *pari passu* with no large oscillations during ripening. The K content of cubiu fruit is due to fertilization and soil type [11,57]. Moreover, variations in the concentration of minerals in fruits are related to the mobility of minerals in the phloem and their translocation trends [58]. These vary according to the species, environmental influences, and growth stage [58]. Thus, similar to other fruits such as papaya (*Carica papaya* L.,

Caricaceae), cubiu mineral content is more affected by the soil fertility, orchard location, and production practice than the ripening process [59]. These facts suggest that chemical composition of plants reflects the chemical composition of the soil and the water in which they grow [60,61,62].

These variations in the macrominerals present pros and cons that should be judiciously considered before cubiu tissue portions can be served to patients. Two scenarios immediately appear. First, macromineral excess could benefit deficient patients (e.g., in the refeeding syndrome and hypokalemia in diabetic patients). Second, the excess could aggravate preexisting mineral overload (e.g., in the chronic kidney disease or CKD).

In the first scenario, malnourished (or starved) patients who are rapidly (re-) fed through a tube or by intravenous route without being given additional P have been known to exhibit not only a deficiency in P but also in K, Mg, and other micronutrients (e.g., vitamins), *i.e.*, refeeding syndrome [13,63,64]. Cubiu tissue portions—especially the ripe pulp and the placenta—could be good supplementary sources of macrominerals for such deficient patients.

In addition, it is possible to regard cubiu fruits as alternative sources of K for hypokalemic diabetic patients because of the high K and the low carbohydrate content of cubiu pulp and placenta throughout ripening. Additionally, there is animal and human experimental evidence demonstrating a hypoglycemic effect of cubiu plant [6]. Fresh banana fruit (e.g., *Musa acuminata* Colla x *Musa balbisiana* Colla, Group AAB, Musaceae) is even richer in K (e.g., 264 mg/100 g) than cubiu fruit, but it has the disadvantage of also being rich in carbohydrates (e.g., 22.3 g/100 g) [65]. Nonetheless, other banana cultivars have lower carbohydrate contents and glycemic indices as well as natural inhibitors of carbohydrate hydrolyzing enzymes (α -amylase, EC 3.2.1.1, and α -glucosidase, EC 3.2.1.20) [66]. This justifies their recommendation in type 2 diabetes mellitus [66].

Hypokalemia, *i.e.*, a serum K^+ less than 3.5 mEq/L, is a common electrolyte imbalance in diabetic patients that leads to substantial morbidity and mortality [67]. Severe hypokalemia, *i.e.*, a serum K^+ less than 2.5 mEq/L, may occur in patients with life-threatening forms of decompensated diabetes mellitus (e.g., diabetic ketoacidosis (DKA), hyperglycemic hyperosmolar syndrome). This is most frequently due to the use of diuretics or nasogastric suction or persistent vomiting [67,68,69,70]. During DKA therapy, serum K^+ further falls as (i) insulin drives K^+ back into the cells, (ii) glucose levels drop, and the decreased osmotic pull allows water and K^+ to move back into cells, and as (iii) acidosis resolves, H^+ moves out of the cells in exchange for K^+ [71]. To conclude, low consumption of potassium-rich foods (e.g., fruits and vegetables) has been reported to contribute in cases of DKA-associated severe hypokalemia [72]. It is another argument that favors the consumption of cubiu fruits in diabetes mellitus.

In the second scenario, knowledge of elevated P levels in ripe cubiu pulp and placenta is of interest due to the pathophysiological implications (e.g., mineral bone disorder syndrome, heart disease) of hyperphosphatemia, *i.e.*, a serum P greater than 4.5 mg/dL in CKD [13,73,74,75]. Importantly, CKD is more common in

subjects with diabetes mellitus [76]. Thus, fruits including cubiu as well as vegetables must be used with caution in dialysis patients to avoid excessive K load [73]. Patients can be advised to boil vegetables to reduce the macromineral content of these foods, *e.g.*, in K and P [73].

4.3. Macronutrient and Caloric Content of Cubiu Fruit Tissues during Ripening

The fruit peel (exocarp) is a protective tissue, and its main functions comprise moderating gas exchange between fruit tissues and the surrounding environment [77]. It protects internal tissues from excessive water loss, mechanical injury, and attack by external weather, diseases, and insects [77]. Fruit peels usually contain higher concentration of dietary fibers and antioxidants than the other tissues [78]. However, cubiu is a rich fruit in soluble fibers (*e.g.*, pectin) both in the peel and in the pulp [1,6,7]. In addition, if pectin was considered in the peel energy calculation, it would have (slightly) decreased the tissue carbohydrate content and, consequently, would have decreased the peel caloric content of cubiu fruit.

Fruit dry matter is nutritionally meaningful, *i.e.*, it is an aggregate of various nutrients and pigments (or bioactive compounds) [79]. The cubiu peel is largely made up of carbohydrates, and it is easy to understand why it has a bittersweet taste when dehydrated as described above. The dry matter is an important quality factor in many crops [80]. It is particularly important for the processing industry, *e.g.*, a large proportion of the pepper produced in the world is used in powder form [81]. However, there is a clear need for further studies on the nutrient (and anti-nutrient) content of fruit peel (in general) and cubiu peel (in particular)—especially during ripening.

The fleshy pulp (mesocarp) is the largest edible portion of cubiu fruit. In contrast to the low overall macronutrient content of whole cubiu fruits (previously mentioned in the introduction), the equivalent levels of the tissue portions were relatively high in this study—especially the lipid content of cubiu pulp. These lipid calories can be valuable in malnourished patients as discussed below. Interestingly, the carbohydrate and energy values of the turning cubiu pulp (6.74 g/100 g fresh weight and 148.47 kJ (35.14 kcal), respectively) were close to those observed in whole camapu fruits (*Physalis angulata* L., Solanaceae) also at the turning stage (*i.e.*, 6.45 g/100 g fresh weight and 152.65 kJ, (36.19 kcal) respectively) [82]. Cubiu fruit pulp is commonly used to prepare high quality, hypocaloric jellies, juices, and other culinary delicacies [6]. Due to its wound healing properties, it is also offered to female patients as blend juice of cubiu and babosa (*Aloe vera* (L.) Burm. f., Asphodelaceae) in the postoperative period of mammoplasty and abdominoplasty in Venezuela.

The placental tissue of fruits belonging to the Solanaceae family has an important metabolic function—especially in the genus *Capsicum* in which the (exclusive) production of capsaicinoids helps protect the developing embryos while offering a pungent pepper taste [9]. Cubiu fruit placenta (endocarp) contains numerous seeds [1]. Seeds are rich in proteins, lipids, and minerals [83]. Thus, cubiu's rich content in ash, proteins, and lipids was partly due to the abundant seed content of this tissue. This rich

macronutrient profile of cubiu seeds was substantiated by two studies [10,57].

The potential caloric contribution of cubiu fruit portions (*e.g.*, pulp and placenta) may be highly desirable—especially in the fasting-related states of distress (*e.g.*, malnutrition, wasting syndrome, such as cancer cachexia) [84,85]. In such morbid states, there is an important protein breakdown, and lipid and carbohydrate calories may exert a suitable nitrogen sparing effect [86]. At the other end of the spectrum of weight disorders, food overconsumption and obesity-related disorders prevail, and monounsaturated fatty acids from fruits are currently recommended [87]. Unsaturated fatty acids predominate in true plant-based diets [88]. Cubiu fruits may be alternative sources to these lipids in such diets [10].

The potential health benefits of tropical fruits have aroused worldwide interest and created space in the international market for exotic fruits [89,90]. By definition, exotic fruits do not originate in the country where they presently occur. Predictably, exotic fruits were once restricted to people living in limited geographic areas, but have now become common in other countries [91]. This growing interest in exotic fruits is in part due to the high prevalence of nutritional disorders (*e.g.*, obesity, metabolic syndrome, diabetes mellitus, and hidden hunger or micronutrient malnutrition), and an urgent need for new therapeutic avenues [90,92]. Accordingly, Brazilian farmers have been cultivating cubiu fruits to export to Japan for the extraction of functional food ingredients such as pectin [7,8].

The concept of panacea dates back to the classical Greek antiquity, but there is no drug or food can cure all illnesses [93]. Thus, eating a varied diet is important [9]. Cubiu is a tropical fruit that is particularly rich in K, low in calories (except for its placental portion at the fully ripe stage), possessing a set of beneficial phytochemicals and other health promoting substances during ripening. It contributes to the human diet when integrated into the nutritional needs of individuals.

5. Conclusions

Three tissue portions of cubiu fruit are most important for human consumption. However, cubiu placenta is the most nutritionally dense tissue portion of the fruit during ripening. Paradoxically, it remains the least studied tissue portion of cubiu fruit. In addition, cubiu fruit possesses an arsenal of phytonutrients and other beneficial molecules making it a promising solanaceous fruit with great potential health benefits throughout the ripening process. In contrast to the low overall macronutrient content of whole cubiu fruit demonstrated previously, the equivalent levels of the tissue portions were relatively high here.

Every nutrient is important in an overpopulated world. Cubiu is rich in ash, proteins, and lipids, especially in the fully ripe placenta, partly because of the abundant seed content of this tissue portion. This caloric contribution may not only replenish energy but also spare nitrogen in malnourished patients. In addition, K was the most abundant macromineral in all tissue portions and ripening stages of cubiu fruit. Considering the high K and the low carbohydrate content of cubiu pulp and placenta during

ripening, it is possible to regard cubiu fruit as an alternative source of K for hypokalemic diabetic patients.

Detailed knowledge of the edible parts of cubiu fruit during ripening will increase its use in health and disease. With this intent, teams of specialists in cubiu fruit from INPA regularly cross the remote villages of the Brazilian Amazonia to teach local people about the healthy properties of this fruit. Furthermore, fruit breeding programs privilege cultivars that are more beneficial to human health. The favorable nutritional characteristics of this cubiu cultivar will promote its use in breeding programs. Nonetheless, there is a clear need for studies using additional analytical approaches to elucidate the phenotypic variation of cubiu fruit weight.

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Conflict of Interest

The authors declare no conflict of interest.

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