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Characterization of carotenoids and color in temperate *Asimina triloba* and comparison to other tropical Annonaceae fruits

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Abstract

Aim: Fruits from the tropical *Annona* genus of family Annonaceae have long been cultivated in tropical Latin America, Africa, and southeastern Asia. *Asimina triloba* is a temperate fruit from Annonaceae, but few comparisons between Annonaceae fruits exist. The objective was to determine how 21 days of refrigerated storage affected the carotenoids and color in ripe and overripe *A. triloba*. A comparison to tropical Annonaceae fruits is provided.

Methods: Pawpaw pulp was stored refrigerated for 21 days. Total carotenoids and β-carotene were determined spectrophotometrically and by HPLC, respectively. C.I.E. L*, a*, and b* values were used to calculate hue angle, chroma, total color change (ΔE), browning index, which have been reported in *A. triloba* previously, and color index, whiteness index, yellowness index, the ratio of a*/b*, and percent change in L*, which are reported herein for the first time.

Results: Overripe pulp contains 5-fold more carotenoids than ripe pulp. A significant decline in total carotenoids was observed during refrigerated storage in the overripe pulp, but not in fresh pulp. At the onset of refrigerated storage, ripe pulp was significantly less brown than overripe pulp, but became more brown during refrigerated storage. No further change in browning was observed during storage of overripe pulp.

Conclusions: Using established conversion factors and the values generated in this study, preliminary indications are that ripe *A. triloba* pulp provides 4.0% (males) and 5.1% (females) of the U.S. Recommended Dietary Allowance (USRDA) of vitamin A for individuals 14 years or older, and overripe provides 3.5% and 4.6%. Carotenoids are well-characterized for *Annona muricata* (*A. muricata*), *Annona reticulata* (*A. reticulata*), and *Annona squamosa* (*A. squamosa*), but these provide less than 1% of the USRDA of vitamin A. A comparison revealed that Annonaceae fruit are nutrient-dense, provide fiber and potassium, are low in fat and protein, and have comparable calcium, iron, magnesium, potassium, and sodium levels. Understanding Annonaceae fruits nutritional value may facilitate increased economic potential.

Keywords

Annonaceae, *Annona*, *Asimina*, nutritional comparison, carotenoids

Introduction

The Annonaceae family, the largest in the order Magnoliales, contains a diverse range of tropical and subtropical fruit-bearing trees and shrubs and is particularly significant in tropical Latin America, where several species are native and have been cultivated for centuries [1]. Only the genera *Annona* and *Asimina* contain species that produce edible fruits. A description of nine species of these genera and their native region is presented in Table 1. The four most widely cultivated and commercially important *Annona* fruits are *Annona muricata* (*A. muricata*), *Annona cherimola* (*A. cherimola*), *Annona squamosa* (*A. squamosa*), and *Annona reticulata* (*A. reticulata*), in that order. These fruits are consumed whole and used in beverages, desserts, and traditional medicine [2]. *Annona coriacea* (*A. coriacea*), *Annona crassiflora* (*A. crassiflora*), *Annona macroprophyllata* (*A. macroprophyllata*), and *Annona purpurea* (*A. purpurea*) are known in their local areas but have not achieved enough commercial popularity to be cultivated on a wide scale.

Table 1. Characteristics of *Asimina* and *Annona* fruits from the Annonaceae family

Scientific name (Common names)	Appearance	Flavor	Size	Native region
Annona cherimola Mill. (Cherimoya, Chirimoya)	Green, scaly skin, creamy white flesh	Sweet, custard-like (banana, pineapple, strawberry)	0.5 to 2 kg	Tropical Andean valleys (Ecuador, Colombia, Peru, Bolivia)
Annona coriacea Mart. (Araticum)	Greenish-yellow skin, fibrous succulent pulp	Sweet, strong aroma	Up to 1.5 kg	South America (Brazil, Paraguay, the Cerrado)
Annona crassiflora Mart. (Marolo)	Green-brownish skin, creamy pulp	Sweet, strong odor, unique flavor	Up to 4.5 kg	South America (Brazil, Paraguay, the Cerrado)
Annona macroprophyllata Donn. Sm. (Anona, Ilama, Papuasa)	Green or pink skin, soft, creamy flesh	Sweet, slightly tangy (pineapple, banana)	0.5 to 1 kg	Tropical Central America (Mexico, Guatemala)
Annona muricata L. (Graviola, Guanabana, Soursop)	Dark green, spiny skin, white fibrous flesh	Sweet and tart (strawberry, pineapple)	Up to 4.5 kg	Tropical Americas (Caribbean)
Annona purpurea Moc. and Sessé ex Dunal (Soncoya)	Rough, brown skin with hooklike projections, stringy pulp	Sweet with a slightly sour undertone	0.5 to 1 kg	Central and South America (Mexico to Venezuela)
Annona reticulata L. (Custard Apple, Anona Roja)	Yellowish-green to brown skin, creamy flesh	Sweet, custard-like, slightly grainy (banana, pineapple)	0.5 to 1 kg	Tropical Americas
Annona squamosa L. (Sugar Apple, Saramuyo)	Green, knobby skin, creamy white flesh	Very sweet, custard-like (banana, pineapple)	0.2 to 0.5 kg	Tropical Americas
Asimina triloba (L.) Dunal (Pawpaw, North American Pawpaw, Indian Banana)	Greenish-yellow skin, custard-like flesh	Sweet, tropical flavor (banana, mango, melon)	Up to 1 kg	Temperate North America (eastern USA)

Asimina triloba (*A. triloba*) is not native to Latin America. Rather, it is the only temperate fruit belonging to the Annonaceae family, and its native growing range is the eastern United States and Canada in USDA plant hardiness zones 5–9 [3]. It is difficult to know how this one species of Annonaceae became native to temperate North America, however, there is some evidence that herbivorous megafauna facilitated seed dispersal and subsequent distribution of flora from Central America lowlands into North America over 10,000 years ago [4]. *A. triloba* is commonly referred to as the pawpaw in North America, so it should not be confused with papaya (*Carica papaya*), which is also referred to as pawpaw in other parts of the Americas and Asia. *A. triloba* is gaining popularity in North America, Europe, and Asia for its unique flavor, but is still a niche crop compared to other fruits [5]. *A. triloba* has a short shelf life due to its susceptibility to brown discoloration and softening that happens easily when lightly bruised or naturally during storage.

Carotenoids are a class of antioxidative pigments responsible for many of the red, orange, and yellow colors in fresh fruits and are known to be health-promoting antioxidants that protect from certain health

conditions and enhance immune response [6]. The provitamin A carotenoids (α-, β-, γ-carotene and β-cryptoxanthin) are nutritionally important because they can be converted into vitamin A in the body and have antioxidant properties [7]. β-Cryptoxanthin is a reddish pigment found in fruits like papaya, tangerines, and red bell peppers [8]. Xanthophylls are a category of carotenoids distinguished from other carotenoids by the presence of oxygen in their structure. Nutritionally important xanthophylls include antheraxanthin, lutein, neoxanthin, violaxanthin, and zeaxanthin. Certain xanthophylls, such as lutein and zeaxanthin, are essential for eye health [9, 10]. Lycopene is a red-pigmented carotenoid known as a strong antioxidant [11] that has been linked to mitigating the risk of certain cancers and heart disease.

Some Annonaceae fruit species have established commercial markets, whereas others remain commercially under-exploited. Thus, there is significant potential for growth and innovation, which could be aided by research on conditions that affect their nutritional content, but few of the studies that report the nutritional information for Annonaceae fruits include an analysis of the carotenoid content. Therefore, the objective of this study was to provide a preliminary carotenoid analysis of *A. triloba* for different ripeness levels (ripe and overripe) during 21 days of refrigerated storage. The study endeavors for the first time to compare the nutritional content of *A. triloba* to commercially relevant fruits from the tropical *Annona* genus.

Materials and methods

All chemicals and reagents were obtained from Fisher Scientific (Waltham, MA) or Sigma-Aldrich (St. Louis, MO). Two lots of ripe and overripe pawpaws (*A. triloba*) were obtained. Whole fruits from a single tree in Athens, Ohio, were obtained in 2022. Fruits from this tree are sometimes referred to as cultivar 'Rana', but this designation is not universally accepted, so it will not be so named in this study. The other lot was a mix of whole fruits from different trees for which the cultivars were not known that were obtained in 2023 from Fox Paw Ridge Farm (Cincinnati, OH). The two lots will be differentiated only by their harvest year (2022, 2023) because genetic homogeneity of the samples was not assessed due to limitations in identifying and utilizing appropriate molecular markers, collecting a statistically significant number of replicates, and performing the necessary genotyping and bioinformatic analyses to determine genetic diversity and relatedness. Because of this and the fact that growing conditions were not standardized, comparisons between harvests would be immaterial.

The fruit pulp from ripe and overripe whole fruits was separated from the skin and seeds and placed in vacuum-sealed bags. Ripeness levels were assessed subjectively because no research-based method has been developed to assess ripeness for pawpaws. Three replicates were randomly assigned to refrigerated storage times of 0, 7, 14, and 21 days. The pulp was refrigerated (4°C) for the requisite time and then stored at –40°C until analyzed.

Pawpaw pulp (1 g) was extracted for total carotenoid determination and β-carotene quantification by mixing with hexane (10 mL), acetone (5 mL), and methanol (5 mL), followed by sonication in the dark for 6 min with a 1-second pulse at 50% amplitude using a Branson Digital Sonifer 450 (Danbury, Connecticut). The sonicated mixture was shaken overnight under refrigeration, after which distilled water was added to enhance the separation of the polar and non-polar fractions via centrifugation. Total carotenoids were measured in the non-polar fraction by monitoring the absorption at 450 nm (Spectronic Genesys 5, Thermo Electron Corporation, Madison, WI) and quantified using the molar extinction coefficient for total carotenoids (135,310 L mol^{-1} cm^{-1}).

Analysis of β-carotene was performed using high-performance liquid chromatography (HPLC). The non-polar extracts described above were evaporated to dryness, the residue dissolved in a known volume of hexane, and the extracts filtered through a 0.2 micron filter. An HPLC (Dionex UltiMate 3000 stack, Germering, Germany) equipped with a four-channel pump, autosampler, and UV-VIS detector was operated using Chromeleon 7.0 software. A reverse phase C30 HPLC column (250 × 4.60 mm) with gradient mobile phase flow of acetonitrile/methanol (90:10) and methanol/ethyl acetate (64:36) was employed, and a detection wavelength of 450 nm quantified β-carotene based on retention time compared to a standard curve of β-carotene.

A calibrated colorimeter (Konica Minolta, NJ) was used to determine C.I.E. L*, a*, and b* values. The applied color values described below were calculated according to established methods [12].

The ratio of a* to b* provides a measure of the relationship between red/green and yellow/blue.

$$Ratio\ of\ a^* \ to\ b^* = \frac{a^*}{b^*}$$

Hue angle measures the specific hue of a color placed in one of four quadrants of a color wheel, with 0° (0 radians) indicating red hue, 90° (π/2 radians) indicating yellow hue, 180° (π radians) indicating green hue, and 270° (3π/2 radians) indicating blue hue.

$$Hue\ angle = arctan\left(\frac{b^*}{a^*}\right)$$

Chroma measures the degree of difference of a hue, with a greater chroma indicating more color.

$$Chroma = \sqrt{(a^*)^2 + (b^*)^2}$$

Total color change (ΔE) measures the magnitude of color difference between samples and a reference point, in this case, the color difference between the day 0 fresh pulp and the overripe and stored pulp.

$$\Delta E = \sqrt{\left(L_0^* - L^*\right)^2 + \left(a_0^* - a^*\right)^2 + \left(b_0^* - b^*\right)^2}$$

Browning index is used as a monitor of changes in browning between samples and a reference point, in this case, the browning difference between the day 0 fresh pulp and the overripe and stored pulp.

$Browning\ index = \frac{100(x - 0.31)}{0.17}$, where $x = \frac{a^* + 1.75L^*}{5.645L^* + a_0^* - 3.01b^*}$ (a_0* is the initial a* value of day 0 fresh pulp).

The percent decrease in L* value represents the degree of browning expressed as the reduction of L* value of the overripe and stored pulp samples compared to a reference point, the day 0 fresh pulp.

$Percent\ decrease\ in\ L^* = \frac{L_a^* - L^*}{L_a^*} \times 100$ (L^*_a is the initial L* value of day 0 fresh pulp).

Whiteness index combines L*, a*, and b* to measure the degree of whiteness of pulp, which indicates the extent of discoloration.

$$Whiteness\ index = 100 - \sqrt{(100 - L^*)^2 + (a^*)^2 + (b^*)^2}$$

Color index measures the saturation of color and is often used for citrus products.

$$Color\ index = \frac{1000 \times a^*}{L^* \times a^*}$$

Yellowness index is a relationship between L* and b* that indicates the degree of yellowness, often used for samples without much color.

$$Yellowness\ index = \frac{142.86 \times b^*}{L^*}$$

Analysis of variance and correlation analysis were performed using IBM SPSS Statistics software (version 29.0, Armonk, NY), with a significance level set at $p < 0.05$ for all tests. Duncan's Multiple Range test determined significant differences between means.

Results

Carotenoid content of *A. triloba*

The β-carotene and total carotenoid content for ripe and overripe *A. triloba* pulp from the 2022 harvest year is shown in Table 2. Overripe pulp contained significantly more total carotenoids than ripe pulp (p = 0.017), but no difference was observed in β-carotene between ripe and overripe pulp.

Table 2. Means ± standard deviation for total carotenoids (µg total carotenoids/100 g pulp), β-carotene (µg β-carotene/100 g pulp), and CIELAB color values (L*, a*, b*) in ripe and overripe *A. triloba* pulp harvested in 2022

Measurement	Ripe	Overripe	*p*-value
Total carotenoids	561 ± 154	947 ± 70	0.017
β-Carotene	426 ± 271	389 ± 184	0.856
L*	79.6 ± 1.2	65.9 ± 3.8	0.008
a*	2.2 ± 2.7	3.8 ± 1.2	0.509
b*	40.2 ± 2.6	38.5 ± 15.4	0.849

A.triloba pulp obtained from a composite of fruits of unknown cultivar during the 2023 harvest was used to explore carotenoid content (µg total carotenoids/100 g pulp) of pawpaw pulp for two main effects, ripeness level and refrigerated storage time. The main effect of ripeness revealed a significant difference in total carotenoids ($p < 0.007$) between ripe and overripe pulp, with overripe pulp containing 5-fold more carotenoids (1,143 µg total carotenoids/100 g pulp) than ripe pulp (230 µg total carotenoids/100 g pulp). The main effect of refrigerated storage time showed a significant decline in total carotenoids ($p = 0.013$), with pawpaw pulp stored for 0 and 7 days exhibiting significantly higher total carotenoids than pulp stored for 21 days. The two-way interactions (Table 3) show that these differences ($p < 0.001$) were driven solely by the decline of total carotenoids in the overripe pulp because total carotenoids in the fresh pulp that was stored refrigerated did not decline significantly during the 21-day storage period. Overripe pulp that was stored for 21 days contained significantly lower total carotenoids (23% to 35%) than overripe pulp that was stored refrigerated for 0, 7, or 14 days. Thus, it appears that more carotenoids are found in overripe pulp than in ripe pulp, but the levels in ripe pulp are stable during refrigerated storage, whereas the levels in the overripe pulp decline during refrigerated storage.

Table 3. Means ± standard deviation for total carotenoids (µg total carotenoids/100 g pulp) and CIELAB color values (L*, a*, b*) and calculated color attributes in *A. triloba* from the 2023 harvest year for ripe and overripe pulp during 21-day refrigerated storage

Measurement	Ripe				Overripe			
	Day 0	Day 7	Day 14	Day 21	Day 0	Day 7	Day 14	Day 21
Total carotenoids	307 ± 114^{d}	243 ± 24^{d}	148 ± 33^{d}	221 ± 68^{d}	1,140 ± 101^{b}	1,353 ± 134^{a}	1,201 ± 149^{ab}	878 ± 190^{c}
L*	78.5 ± 0.4^{a}	42.7 ± 2.1^{de}	40.5 ± 1.1^{de}	39.1 ± 0.4^{e}	65.5 ± 1.7^{b}	47.9 ± 3.7^{c}	47.4 ± 1.3^{c}	43.3 ± 3.1^{d}
a*	1.6 ± 0.7^{c}	16.3 ± 0.9^{a}	17.2 ± 0.7^{a}	15.6 ± 0.3^{a}	10.8 ± 0.4^{b}	15.0 ± 5.9^{ab}	17.1 ± 1.3^{a}	15.7 ± 3.0^{a}
b*	22.2 ± 1.0^{d}	29.0 ± 1.1^{bc}	29.3 ± 1.1^{bc}	22.3 ± 0.5^{cd}	41.3 ± 1.3^{a}	28.7 ± 6.2^{bc}	32.9 ± 1.6^{b}	27.2 ± 3.3^{abc}
Ratio a*/b*	0.07 ± 0.03^{d}	0.57 ± 0.05^{ab}	0.59 ± 0.01^{ab}	0.70 ± 0.30^{a}	0.26 ± 0.02^{c}	0.51 ± 0.11^{b}	0.52 ± 0.06^{b}	0.58 ± 0.13^{ab}
Hue angle	85.9 ± 1.8^{a}	60.6 ± 2.2^{cd}	59.5 ± 0.6^{cd}	54.6 ± 7.2^{d}	75.2 ± 1.2^{b}	63.0 ± 5.2^{c}	62.6 ± 2.7^{c}	60.1 ± 5.6^{cd}
Chroma	22.3 ± 1.0^{d}	33.3 ± 0.7^{bc}	34.0 ± 1.2^{bc}	27.4 ± 2.3^{cd}	42.7 ± 6.6^{a}	32.5 ± 8.0^{bc}	37.1 ± 1.2^{ab}	31.5 ± 3.3^{bc}
Total color change (ΔE)	-	39.4 ± 1.9^{ab}	41.7 ± 0.6^{a}	41.9 ± 0.4^{a}	25.2 ± 5.0^{c}	34.4 ± 6.8^{b}	36.4 ± 1.6^{ab}	38.4 ± 3.7^{ab}
Browning index	33.6 ± 2.6^{c}	160.8 ± 7.2^{ab}	180.2 ± 3.5^{a}	136.5 ± 22.2^{ab}	116.5 ± 24.5^{b}	139.5 ± 73.7^{ab}	162.2 ± 14.8^{ab}	145.3 ± 27.5^{ab}
L* decrease (%)	-	45.6 ± 2.6^{ab}	48.4 ± 1.3^{ab}	50.2 ± 1.3^{a}	16.7 ± 1.5^{d}	38.9 ± 4.7^{c}	39.6 ± 1.7^{c}	44.8 ± 3.9^{b}
Whiteness index	69.0 ± 1.0^{a}	33.7 ± 1.5^{cd}	31.5 ± 0.3^{d}	33.2 ± 1.8^{cd}	44.9 ± 4.9^{b}	38.5 ± 7.4^{c}	35.6 ± 1.7^{cd}	35.1 ± 3.0^{cd}
Color index	0.09 ± 0.4^{c}	1.33 ± 0.2^{b}	1.45 ± 0.5^{ab}	1.84 ± 0.5^{a}	0.40 ± 0.4^{c}	1.08 ± 0.3^{b}	1.10 ± 0.1^{b}	1.36 ± 0.4^{b}
Yellowness index	40.5 ± 1.9^{b}	97.0 ± 1.1^{a}	103.3 ± 1.6^{a}	81.9 ± 15.8^{a}	90.2 ± 13.9^{a}	86.9 ± 26.1^{a}	99.3 ± 6.8^{a}	89.9 ± 9.5^{a}

$^{a–e}$ Different superscripts within a row indicate significant differences between the means for that attribute ($p < 0.05$)

Color of *A. triloba* affected by ripeness and refrigerated storage

CIELAB L*, a*, b* values were measured for *A. triloba* harvested in 2022 (Table 2) and 2023 (Table 3). These values were used to calculate applied color attributes, of which color index, whiteness index, yellowness index, the ratio of a*/b*, and percent change in L* are reported for *A. triloba* for the first time. A statistical comparison between fresh ripe pulp for the two harvest years was undertaken and shows that pulp color is not homogenous. This observation is not surprising when considering that qualitative descriptions of pawpaw pulp from different cultivars almost always note a range of descriptors such as creamy white, bright yellow, or shades of orange [13]. The 2022 *A. triloba* pulp, obtained from fruit from a single tree, was statistically more yellow (higher b*, $p < 0.001$) and exhibited a correspondingly higher chroma ($p < 0.001$) and yellowness index ($p < 0.001$) than the 2023 pulp, which was a composite of different cultivars grown at a different location than the 2022 pulp. The ripe pulp from 2022 *A. triloba* was also more brown, as indicated by a higher browning index ($p = 0.007$) and a correspondingly lower whiteness index ($p = 0.001$) than the 2023 pulp.

There were many significant indicators that suggested that overripe pulp in *A. triloba* harvested in 2023 (Table 3) exhibited browning compared to ripe pulp, including lower L* value and whiteness and yellowness indices, and higher browning and total color indices between the overripe and ripe pulp. Browning index significantly increased in ripe pulp after day 0, but no further increase was observed during subsequent refrigerated storage. In overripe pulp, in which significant browning was already indicated at the onset of refrigerated storage, no further change in browning index was observed. Significantly lower L* value was also observed in overripe pulp compared to ripe pulp in *A. triloba* harvested in 2022 (Table 2).

Relationship between carotenoids, color, ripeness, and refrigerated storage

Pearson correlation coefficients were computed from the 2023 *A. triloba* to assess the linear relationship between the applied color attributes and total carotenoids, ripeness level, and storage. There was a significant but moderately positive correlation between total carotenoids and both b* [$r(22) = 0.515$, $p = 0.010$] and chroma [$r(22) = 0.482$, $p = 0.017$], although a* was not significantly correlated with total carotenoids. Regardless, this result agrees with the literature that shows that positive correlations between carotenoids and color values, for example, in nectarines, where the association of red and yellow color was directly correlated with increased total carotenoid content that was independent of the initial white or yellow color of the pulp [14].

The correlations between ripeness and both b* and chroma, but not a*, mirror those for total carotenoids, with significant but moderately positive correlations for b* [$r(22) = 0.527$, $p = 0.008$] and chroma [$r(22) = 0.510$, $p = 0.011$]. The significant, strong, positive correlation between total carotenoids and ripeness [$r(22) = 0.945$, $p < 0.001$] echoes the results presented previously that indicate that total carotenoids increase as *A. triloba* becomes more ripe.

The Pearson correlation coefficients that were computed to assess the linear relationship between storage time and each of the 12 applied color attributes (Table 3) of *A. triloba* indicate that pulp color during storage is significantly correlated to a change in 9 of the 12 attributes, some of which are indicators of pulp browning. There were significant, positive correlations between storage time and color index [$r(22) = 0.806$, $p < 0.001$], percent L* reduction [$r(22) = 0.795$, $p < 0.001$], a*/b* ratio [$r(22) = 0.779$, $p < 0.001$], ΔE [$r(22) = 0.706$, $p < 0.001$], a* [$r(22) = 0.625$, $p < 0.001$], and browning index [$r(22) = 0.498$, $p < 0.013$]. There were significant, negative correlations between storage time and L* [$r(22) = -0.795$, $p < 0.001$], hue angle [$r(22) = -0.779$, $p < 0.001$], and whiteness index [$r(22) = -0.667$, $p < 0.001$].

The positive correlations between storage time and both the percent L* reduction and browning index, as well as the negative correlations between storage time and both L* and whiteness index, indicate that the color of the pulp becomes darker or more brown as the pulp is stored. Several factors can contribute to the darkening or browning of the pulp, but the major factor is probably enzymatic browning, where polyphenol oxidases catalyze the oxidation of phenolic compounds. It has been shown that polyphenol oxidase is active in pawpaw pulp [15], and the phenolic compounds that are substrates for polyphenol oxidase are present in the pulp [16].

Discussion

Understanding carotenoids in Annonaceae fruit pulp

Vitamin A is reported on most nutrition labels worldwide in retinol activity equivalents (RAE) or international units (IU). RAE conversion factors have been established for β-carotene (12 µg to 1 µg RAE) and total carotenoids (24 µg to 1 µg RAE), although the total carotenoid conversion factor relies on the assumption that the majority of total carotenoids are composed of dietary provitamin A carotenoids such as α-carotene and β-cryptoxanthin [17]. Using these conversion factors, *A. triloba* contains 36 µg RAE per 100 g edible pulp based on β-carotene and 23 µg RAE per 100 g edible pulp based on total carotenoids. The latter value probably should be used with caution because individual carotenoids of *A. triloba* have not been identified, so it is not known if this value actually reports the RAE solely of dietary provitamin A compounds. *A. muricata*, for example, contains carotenoids that are not classified as dietary provitamin A, such as lycopene and lutein. The same *A. triloba* pulp that was obtained from obviously overripe fruit during the 2022 harvest contained 389 µg β-carotene and 947 µg total carotenoids per 100 g edible pulp (Table 2), converting to 32 µg RAE and 39 µg RAE per 100 g edible pulp, respectively.

Using the 120 g serving size that has been established for *A. triloba* [18] and the seemingly more reliable conversion using β-carotene, ripe pulp provides 4.0% (males) and 5.1% (females) of the U.S. Recommended Dietary Allowance (USRDA) of vitamin A for individuals 14 years or older, and overripe *A. triloba* pulp provides 3.5% (males) and 4.6% (females) of the USRDA of vitamin A for this population. For comparison, a serving of mango provides 10% (males) and 13% (females), and a serving of banana provides less than 1% of the USRDA of vitamin A for these populations.

Annonaceae fruits are climacteric, and an increase in carotenoids in other climacteric fruits agrees with research that has shown that typical post-climacteric biochemical changes were not linked to post-climacteric carotenoid losses [19]. This research showed that carotenoids, especially lutein, were stable in the overripe stage [19] of nectarines [*Prunus persica* (L.) Batsch], hybrid persimmons (*Diospyros virginiana* L. × *Diospyros kaki* L.f.), and bananas (*Musa acuminata* Colla). Other research was similar. In the 'red banana' cultivar of bananas [20], yellow mombin fruit (*Spondias mombin* L.*)* of unknown cultivar [21], and mango (*Mangifera indica* L.*)* of unknown cultivar [22], higher carotenoid levels were found in fruits that were 4, 9, and 16 days post-climacteric, respectively.

A comparison of the carotenoid content of *A. triloba* and seven *Anona* spp. is shown in Table 4. The individual carotenoids in Annonaceae fruit have been well-characterized in *A. muricata*, *A. reticulata*, and *A. squamosa*, with 10, 8, and 8 individual carotenoids reported in the literature, respectively. The five other Annonaceae species, *A. triloba*, *A. cherimola*, *A. coriacea*, *A. crassiflora*, and *A. purpurea*, are not as thoroughly studied, with only 1–3 individual carotenoids reported in the literature for each. The provitamin A carotenoids (α-, β-, γ-carotene and β-cryptoxanthin) are nutritionally important because they can be converted into vitamin A in the body and have antioxidant properties [7]. β-Carotene has been quantified in *A. triloba* and six of the seven Annonaceae species, with values ranging from 0.19 to 1,970 µg per 100 g pulp (in *A. muricata* and *A. crassiflora*, respectively). The five species for which α-carotene has been quantified indicate that *A. reticulata*, *A. squamosa*, and *A. crassiflora* [23] have higher levels of α- compared to β-carotene [24], whereas *A. cherimola* [25] have higher levels of β- compared to α-carotene. The research on *A. muricata* is equivocal, with a study showing higher levels of β- compared to α-carotene [26] and another showing the reverse [24].

Using the conversion values for individual provitamin A carotenoids described previously [17] and assuming a 100 g serving size as a basis, the percentage per serving of the USRDA of vitamin A for individuals 14 years or older for the four most widely cultivated and commercially important *Annona* species ranges from: *A. muricata* (0.01–0.79% males, 0.01–1.01% females), *A. cherimola* (0.02% males, 0.03% females), *A. squamosa* (0.09% males, 0.12% females), and *A. reticulata* (0.10% males, 0.13% females). This suggests that *Annona* species do not provide significant dietary vitamin A on their own or compared to *A. triloba. Annona* species also are a source of xanthophylls, but do not contain high enough

Table 4. Carotenoid content (per 100 g edible fruit) of eight Annonaceae fruits

Carotenoid	*Asimina triloba* (L.) Dunal	*Annona cherimola* Mill.	*Annona coriacea* Mart.	*Annona crassiflora* Mart.	*Annona muricata* L.	*Annona purpurea* Moc. and Sessé ex Dunal	*Annona reticulata* L.	*Annona squamosa* L.
Provitamin A carotenoids								
α-Carotene (μg)	NR	ND[9]	NR	2,980[2]	2[4], 8.5[5], ND[9]	NR	8.76[5]	10.45[5]
β-Carotene (μg)	426*, 82[7]	2[9], ND[1]	520[8]	1,970[2]	5[4], 1[9], 0.19[5]	NR	5.77[5]	3.82[5]
γ-Carotene (μg)	NR	NR	NR	NR	1.30[5]	NR	0.93[5]	1.74[5]
β-Cryptoxanthin (μg)	NR	1[9], ND[1]	NR	NR	5[4], ND[9]	NR	NR	NR
Xanthophylls								
Antheraxanthin (μg)	NR	NR	NR	NR	0.09[5]	NR	1.06[5]	0.20[5]
Lutein (μg)	NR	ND[1]	NR	NR	6[4], 2.86[5]	230[6]	6.62[5]	1.14[5]
Lutein + Zeaxanthin (μg)	NR	6[9]	NR	NR	ND[9]	NR	NR	NR
Neoxanthin (μg)	NR	NR	NR	NR	0.68[5]	NR	0.59[5]	0.68[5]
Violaxanthin (μg)	NR	NR	NR	NR	0.33[5]	NR	0.09[5]	12.44[5]
Zeaxanthin (μg)	NR	NR	NR	NR	1.09[5], ND[4]	680[6]	1.37[5]	10.45[5]
Uncategorized carotenoids								
Lycopene (μg)	NR	ND[9]	340[8]	20[2]	8[4], ND[9]	NR	NR	NR
Retinol (μg)	NR	ND[9]	NR	NR	ND[9]	ND[9]	NR	ND[9]
Vitamin A (IU)	NR	5[9]	NR	5,776[2]	2[9]	NR	33[9]	6[9]
Vitamin A (RAE)	NR	ND[9]	NR	289[2]	ND[9]	NR	2[9]	ND[9]
Total carotenoids (μg)	561*, 307**	29[3]	NR	4,980[2]	NR	4,100[6]	NR	NR

"NR" indicates that the nutrient was not reported; "ND" indicates the nutrient was analyzed but was not detected at or above the threshold level. [1]Albuquerque et al. (2016) [27]; [2]de Morais Cardoso et al. (2013) [23]; [3]Gentile et al. (2020) [28]; [4]Isabelle et al. (2010) [26]; [5]Chimbevo et al. (2017) [24]; [6]Murillo et al. (2010) [29]; [7]Nam et al. (2018) [30]; [8]Schiassi et al. (2018) [31]; [9]USDA [25]; *values from the current study (2022 harvest year); **values from the current study (2023 harvest year)

levels of lutein and zeaxanthin to meet the daily recommendation of these carotenoids for eye health [9, 10]. The presence of lycopene, which is neither a provitamin A carotenoid nor a xanthophyll, was reported only in *A. muricata* [24].

Color comparison of Annonaceae fruit pulp

The C.I.E. color value data for different cultivars of *A. triloba*, *A. cherimola*, *A. diversifolia*, and *A. muricata* are shown in Table 5. The data were obtained from different studies and reinforce the finding that color values for *A. triloba* cultivars are not homogenous. However, this observation probably should not be universally applied to other Annonaceae fruits. The most obvious Annonaceae fruit that is non-homogenous in color is *A. diversifolia*, reported in the literature and on Table 5 as varieties labeled white, pink, and deep pink [32], which probably come from cultivars 'Annona Blanca', 'Fairchild' or 'Rosendo Pérez', and 'Genova Red', respectively. Not surprisingly, the color values are directional from white to deep pink with notably increasing redness (a*). *A. muricata* and *A. cherimola* redness (a*) and (b*) color values are relatively uniform, suggesting homogeneity, although there are some differences in lightness (L*).

Table 5. CIELAB color values (L*, a*, b*) from cultivars of Annonaceae fruit pulp from various studies

Species	Cultivar	L*	a*	b*	Study
A. cherimola Mill.	Campas	78.4	–1.1	15.9	Gentile et al. (2020) [28]
	Chaffey	82.8	–0.9	13.7	Gentile et al. (2020) [28]
	Daniela	81.4	–1.1	14.6	Gentile et al. (2020) [28]

Table 5. CIELAB color values (L*, a*, b*) from cultivars of Annonaceae fruit pulp from various studies (*continued*)

Species	Cultivar	L*	a*	b*	Study
	Fino de Jete	83.5	–1.1	13.0	Gentile et al. (2020) [28]
	Torre1	82.0	–1.5	12.0	Gentile et al. (2020) [28]
	Torre2	82.3	–0.9	13.9	Gentile et al. (2020) [28]
	White	81.3	–0.8	13.3	Gentile et al. (2020) [28]
A. macroprophyllata Donn. Sm.	White	65.1	2.3	17.8	Julián-Loaeza et al. (2011) [32]
	Pink	58.1	6.7	10.7	Julián-Loaeza et al. (2011) [32]
	Deep pink	47.4	18.9	–0.1	Julián-Loaeza et al. (2011) [32]
A. muricata L.	Unknown	64.3	–4.6	12.8	Tran et al. (2020) [33]
	Elita	89	–1	13	Márquez Cardozo et al. (2012) [34]
A. triloba (L.) Dunal	Sunflower	64.8	–2.8	27.8	Brannan (2016) [15]
	Sunflower	85.0	–1.4	45.0	Lolletti et al. (2021) [35]
	NC-1	79.8	2.2	47.8	Lolletti et al. (2021) [35]
	NC-1	77.1	10.1	45.9	Brannan et al. (2015) [16]
	Shenandoah	74.4	9.3	49.4	Brannan et al. (2021) [18]
	Shenandoah	64.3	6.3	39.8	Zhang et al. (2017) [36]
	Shenandoah	72.1	7.6	45.5	Adainoo et al. (2022) [37]

A. cherimola: *Annona cherimola*; *A. macroprophyllata*: *Annona macroprophyllata*; *A. muricata*: *Annona muricata*; *A. triloba*: *Asimina triloba*

Comparison of nutritional composition of Annonaceae fruit pulp

As shown in Table 6, there are many nutritional similarities between the nutritional content of six Annonaceae fruits that can be found in research or a database. Detailed nutritional information is reported in the literature for *A. triloba* [18, 30] and *A. macroprophyllata* [32], and nutritional information for the four most widely cultivated Annona species, *A. muricata*, *A. cherimola*, *A. squamosa*, *and A. reticulata*, is reported in the U.S. FoodData Central database [25]. All species have a caloric content between 276–423 kJ (66–101 kcal) per 100 g edible fruit, ≤ 1% ash and lipid, and around 1% protein [18, 25, 30, 32]. There is some variation between species for total carbohydrates and moisture.

Table 6. Nutrition comparison (per 100 g edible fruit) of six Annonaceae fruits

Nutritional citation	***Asimina triloba* (L.) Dunal**	***Asimina triloba* (L.) Dunal**	***Annona cherimola* Mill.**	***Annona macroprophyllata* Donn. Sm.**	***Annona muricata* L.**	***Annona reticulata* L.**	***Annona squamosa* L.**
	Brannan et al. (2021) [18]	**Nam et al. (2018) [30]**	**USDA [25]**	**Julián-Loaeza et al. (2011) [32]**	**USDA [25]**	**USDA [25]**	**USDA [25]**
Proximates							
Calories (kcal)	85	84	84	76	66	101	94
Calories (kJ)	357	353	351	318	276	423	393
Moisture (g)	74.5	79.1	79.4	79	81.2	71.5	73.2
Protein (g)	0.7	1.5	1.6	1	1	1.7	2.1
Total lipid (g)	0.6	0.4	0.7	ND	0.3	0.6	0.3
MUFA (g)	0.05	0.06	0.055	NR	0.09	NR	0.114
PUFA (g)	ND	0.06	0.188	NR	0.069	NR	0.04
Saturated FA (g)	ND	0.05	0.233	NR	0.051	0.231	0.048
Trans FA (g)	ND	NR	ND	NR	ND	ND	ND
Cholesterol (mg)	ND	NR	ND	NR	ND	NR	ND
Ash (g)	0.4	0.4	0.65	NR	0.7	1	0.78
Carbohydrates (g)	23.8	18.6	17.7	17	16.8	25.2	23.6

Table 6. Nutrition comparison (per 100 g edible fruit) of six Annonaceae fruits (*continued*)

Nutritional citation	*Asimina triloba* (L.) Dunal	*Asimina triloba* (L.) Dunal	*Annona cherimola* Mill.	*Annona macroprophyllata* Donn. Sm.	*Annona muricata* L.	*Annona reticulata* L.	*Annona squamosa* L.
	Brannan et al. (2021) [18]	Nam et al. (2018) [30]	USDA [25]	Julián-Loaeza et al. (2011) [32]	USDA [25]	USDA [25]	USDA [25]
Dietary fiber (g)	4.5	5.8	3	2	3.3	2.4	4.4
Total sugars (g)	16.3	13.1	NR	15*	13.5	NR	NR
Sucrose (g)	11.4	9.3	NR	8	NR	NR	NR
Glucose (g)	2.7	2.1	NR	3	NR	NR	NR
Fructose (g)	2.2	1.7	NR	4	NR	NR	NR
Lactose (g)	ND	ND	NR	NR	NR	NR	NR
Maltose (g)	ND	ND	NR	NR	NR	NR	NR
Vitamins							
Vitamin C (mg)	4.92	1	12.6	2	20.6	19.2	36.3
Vitamin D (IU)	ND	ND	NR	NR	NR	NR	NR
Vitamin E (mg)	NR	NR	0.27	NR	0.08	NR	NR
Thiamin (mg)	NR	ND	0.1	NR	0.07	0.08	0.11
Riboflavin (mg)	NR	ND	0.13	NR	0.05	0.1	0.11
Niacin (mg)	NR	ND	0.64	NR	0.9	0.5	0.88
B5 (mg)	NR	NR	0.35	NR	0.25	0.14	0.23
B6 (mg)	NR	NR	0.26	NR	0.06	0.22	0.2
Folate (µg)	NR	NR	23	NR	14	NR	14
Minerals							
Calcium (mg)	13	8	10	13	14	30	24
Copper (mg)	NR	ND	0.07	NR	0.09	NR	0.09
Iron (mg)	0.2	0.3	0.27	NR	0.6	0.71	0.6
Magnesium (mg)	NR	11	17	12	21	18	21
Manganese (mg)	NR	NR	0.09	NR	NR	NR	NR
Phosphorus (mg)	NR	NR	26	NR	27	21	32
Potassium (mg)	201	239	287	344	278	382	247
Sodium (mg)	1	ND	7	3	14	4	9
Sulfur (mg)	NR	NR	NR	NR	NR	NR	NR
Zinc (mg)	NR	ND	0.16	9	0.1	NR	0.1
Essential amino acids							
Histidine (mg)	NR	44	21	NR	NR	NR	NR
Isoleucine (mg)	NR	13	42	NR	NR	NR	NR
Leucine (mg)	NR	38	63	NR	NR	NR	NR
Lysine (mg)	NR	30	42	NR	6	37	55
Methionine (mg)	NR	ND	21	NR	7	4	7
Phenylalanine (mg)	NR	28	42	NR	NR	NR	NR
Threonine (mg)	NR	24	52	NR	NR	NR	NR
Tryptophan (mg)	NR	ND	31	NR	11	NR	1
Valine (mg)	NR	24	63	NR	NR	NR	NR
Non-essential amino acids							
Alanine (mg)	NR	67	63	NR	NR	NR	NR
Arginine (mg)	NR	ND	31	NR	NR	NR	NR
Asparagine (mg)	NR	NR	NR	NR	NR	NR	NR

Table 6. Nutrition comparison (per 100 g edible fruit) of six Annonaceae fruits (*continued*)

Nutritional citation	*Asimina triloba* (L.) Dunal	*Asimina triloba* (L.) Dunal	*Annona cherimola* Mill.	*Annona macroprophyllata* Donn. Sm.	*Annona muricata* L.	*Annona reticulata* L.	*Annona squamosa* L.
	Brannan et al. (2021) [18]	Nam et al. (2018) [30]	USDA [25]	Julián-Loaeza et al. (2011) [32]	USDA [25]	USDA [25]	USDA [25]
Aspartic acid (mg)	NR	47	105	NR	NR	NR	NR
Cystine (mg)	NR	ND	1	NR	NR	NR	NR
Glutamic acid (mg)	NR	58	199	NR	NR	NR	NR
Glutamine (mg)	NR	NR	NR	NR	NR	NR	NR
Glycine (mg)	NR	29	52	NR	NR	NR	NR
Proline (mg)	NR	163	157	NR	NR	NR	NR
Serine (mg)	NR	35	63	NR	NR	NR	NR
Tyrosine (mg)	NR	16	31	NR	NR	NR	NR

"NR" indicates that the nutrient was not reported in the analysis; "ND" indicates the nutrient was analyzed but was not detected at or above the threshold level. MUFA: mono unsaturated fatty acids; PUFA: poly unsaturated fatty acids; FA: fatty acids; *: average of three cultivars

From the period spanning 1982–2018, *A. triloba* fruit pulp was mistakenly reported to be a complete protein, i.e., a food that contains all nine essential amino acids. This misinterpretation was due to the fact that the research that reported this finding in 1982 was conducted on *A. triloba* fruit pulp and the skin [38], which is generally considered inedible. Recent research (Table 6) now indicates that *A. triloba* fruit pulp is probably deficient in methionine and tryptophan [30]. No studies could be located in which a complete amino acid profile was conducted on any of the *Annona* species, so no conclusion can be made as to whether any of the *Annona* fruits are complete proteins.

Total carbohydrates (Table 6) ranged from 16.8% to 25.2% across the six species [18, 25, 30, 32]. In *A. triloba*, two nutritional studies reported carbohydrate contents of 18.6% [30] and 23.8% [18]. These two studies also reported higher dietary fiber (5.8% and 4.5%, respectively) than the 2% to 4.4% range reported for five *Annona* species [25, 32]. Annonaceae fruits have 13–16% total sugar, composed of sucrose, glucose, and fructose. However, the distribution of these three simple sugars varies between *A. triloba* and *A. macroprophyllata*, the only *Annona* species for which this data is reported. In *A. triloba*, the distribution of sucrose, glucose, and fructose is 70%, 17%, and 13%, respectively, whereas *A. macroprophyllata* has less sucrose, with a distribution of 53%, 20%, and 27%, respectively. It is not known if this result can be generalized to all *Annona* fruits until such a time as the sugar profile of other *Annona* fruits is reported.

Comparisons between the six *Annona* species can be made for vitamin C and five minerals (calcium, iron, magnesium, potassium, and sodium) because all six species have reported values for these nutrients. With respect to vitamin C, three tiers emerged. At the high end, *A. squamosa* contains vitamin C at an equivalent level to that of mango. *A. muricata* and *A. reticulata* contain about 45% less, and *A. cherimola* about 66% less. *A. triloba* and *A. macroprophyllata* have very little vitamin C. All Annonaceae species contained comparable amounts of calcium, iron, magnesium, potassium, and sodium, which were on par with a serving of mango.

Summary and implications

With respect to carotenoids, higher levels were found in overripe *A. triloba* pulp than in ripe pulp, and the levels in ripe pulp were stable during refrigerated storage, whereas the levels in the overripe pulp declined during refrigerated storage. The significant correlation between total carotenoids and ripeness reinforces that total carotenoids increase as *A. triloba* becomes more ripe. The individual carotenoids in Annonaceae fruit have been well-characterized in *A. muricata*, *A. reticulata*, and *A. squamosa,* but not in other Annonaceae species. *A. reticulata*, *A. crassiflora*, and *A. squamosa* have higher levels of α- compared to β-

carotene, whereas *A. cherimola* has higher levels of β- compared to α-carotene. The research on *A. muricata* is inconclusive.

An interesting finding from this study is the nutritional similarity of *A. triloba,* unique in the family Annonaceae because it is the only temperate genus, and the "normal" *Annona* species that are native to Latin America. Overall, Annonaceae fruits are nutrient-dense and provide a good amount of fiber and potassium, while being low in fat and protein. These fruits do not appear to be complete proteins, although studies are limited, for which a complete amino acid profile would provide confirmation. The carbohydrates from Annonaceae fruits come primarily from the sugars sucrose, glucose, and fructose, albeit with differing distributions. Fruits from some Annonaceae, such as *A. squamosa*, have a good amount of vitamin C, while others, such as *A. triloba* and *A. macroprophyllata*, have very little vitamin C. Fruits from all Annonaceae species contained comparable amounts of calcium, iron, magnesium, potassium, and sodium.

Promotion and marketing of the vitamins and other antioxidative compounds in Annonaceae fruit pulp would enrich their culinary scope and potential. Added benefits would include improving the shelf life and stability of products to which they are incorporated and a reduction in oxidative stress and the associated lower risk of chronic diseases in those who consume them. In addition to their health benefits, the unique flavor and texture of Annonaceae fruits add versatility for creative culinary applications that may encourage healthier eating habits and make delicious, nutritious foods more accessible.

This study has implications with respect to increased utilization of Annonaceae fruit. It would be beneficial if overripe fruits, often discarded, could be utilized in some fashion, but more research on the acceptability of products prepared from overripe pulp is needed. It would also be beneficial to know if other nutrients in addition to carotenoids are stable during refrigerated storage, because this research suggests that if the quality of the fruit can be maintained during refrigerated storage, the carotenoid content also will be maintained. Because the findings from this study apply only to *A. triloba*, further study would be required to determine whether these findings are universal for all Annonaceae fruits, which would add value to Annonaceae fruits.

Conclusion

The pawpaw research presented herein is preliminary in nature, mirroring the post-harvest research on Annonaceae, which is far behind in maturity than many other commercial fruit crops. Strict genetic control in research involving Annonaceae fruit is rarely undertaken, as was the case in this research. Because of this limitation, observed differences in the measured variables could be attributable to inherent genetic variations among individual pawpaw fruit rather than solely the experimental conditions, which could affect the generalizability of the findings. Future research that incorporates controlled experiments to specifically address the genetic background of Annonaceae samples would be important for more robust and generalizable conclusions.

Nonetheless, the results of this study underscore the importance of considering both ripeness and storage duration when evaluating the nutritional content in Annonaceae fruits. The stability of *A. triloba* carotenoids during refrigerated storage suggests that overripe pulp could be utilized in meal prep and food production, which offers practical solutions for both consumer health and sustainable practices in food manufacturing. However, the sensory attributes of overripe fruit pulp, i.e., color, texture, and flavor, will have to be taken into consideration before any generalizations can be made.

This study seeks to enhance the global understanding of the nutritional relevance of Annonaceae fruits, both temperate and tropical, especially in areas where these two climates exist in close proximity. For example, the diverse range of climates in Latin America suggests that *A. triloba* cultivars could be selected and introduced into areas near the native range of tropical Annonaceae, such as the Pampas region of Argentina or temperate regions of central Chile, Uruguay, and southern Brazil. Understanding Annonaceae fruits in this regional context may reveal previously hidden industrial potential and stimulate new awareness that contributes to enhancing local economies.

Abbreviations

HPLC: high-performance liquid chromatography

RAE: retinol activity equivalents

USRDA: U.S. Recommended Dietary Allowance

ΔE: total color change

Declarations

Author contributions

RB: Conceptualization, Investigation, Formal analysis, Methodology, Project administration, Writing—original draft, Writing—review & editing. DON: Investigation, Formal analysis, Funding acquisition, Methodology, Writing—original draft, Writing—review & editing. MJ: Investigation, Methodology, Writing—review & editing.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Ethical approval

Not applicable.

Consent to participate

Not applicable.

Consent to publication

Not applicable.

Availability of data and materials

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

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